VOLUME 43 NUMBER 4, PART II APRIL, 1957

AMERICAN JOURNAL OF OPHTHALMOLOGY

THIRD SERIES FOUNDED BY EDWARD JACKSON

* * * *

The Symposium

on

VIRAL KERATOCONJUNCTIVITIS

September 7 and 8, 1956

Sponsored by the National Institutes of Health

* * * *

For a complete table of contents see pages one and two

Subscription price in United States twelve dollars yearly. In Canada and foreign countries fourteen dollars. Published monthly by the Ophthalmic Publishing Company. Subscription and Advertising Office: 664 North Michigan Avenue, Chicago 11, Illinois. Entered as second class matter at the post office at Menasha, Wisconsin. Printed in U.S.A.

Participants

Virologists and Epidemiologists

Beale, A. J., *University of Toronto*
Bell, J. A., *National Institutes of Health*
*Cheever, F. S., *University of Pittsburgh*
*Cockburn, T. A., *World Health Organization, Ceylon*
Fowle, Ann M. C., *University of Toronto*
Hanna, L., *University of California*
Huebner, R. J., *National Institutes of Health*
Jawetz, E., *University of California*
Korns, R. F., *State of New York Department of Health*
Lennette, E. H., *State of California Department of Health*
Rake, G. W., *University of Pittsburgh*
Sanders, M., *University of Miami*
Scott, T. F. McNair, *University of Pennsylvania*
Snyder, J. C., *Harvard University School of Public Health*

Ophthalmologists

*Bietti, G. B., *University of Rome*
Braley, A. E., *University of Iowa*
*Hogan, M. J., *University of California*
Kimura, S. J., *University of California*
Leopold, I. H., *University of Pennsylvania*
*Mitsui, Y., *Kumamoto University Medical College*
Ormsby, H. L., *University of Toronto*
*Pillat, A., *First University Eye Clinic of Vienna*
Tanaka, C., *Visiting Scientist, National Institutes of Health*
Thygeson, P., *University of California*

* In absentia.

AMERICAN JOURNAL OF OPHTHALMOLOGY

SERIES 3 · VOLUME 43 · NUMBER 4 · PART II · APRIL, 1957

and the *Proceedings* of the Symposium on

VIRAL KERATOCONJUNCTIVITIS

San Francisco, California, September 7 and 8, 1956
Sponsored by the National Institutes of Health

CONTENTS

Herpetic keratoconjunctivitis and keratitis

SYMPOSIUM ON VIRAL KERATOCONJUNCTIVITIS

September 7 and 8, 1956, San Francisco

PARTICIPANTS IN THE SYMPOSIUM, MEMBERS OF THE STUDY SECTIONS, AND ALL OTHER GUESTS:

It is a great pleasure for me as chairman of the Department of Ophthalmology to welcome you to this symposium on viral keratoconjunctivitis.

Viral disease is of ever-increasing importance in ophthalmology and we feel privileged indeed that this important symposium, under the sponsorship of the National Institutes of Health, should be held here. We are particularly honored to have so many famous virologists and epidemiologists among us for a few days and feel sure the results of your deliberations will be of very great value to ophthalmology.

May I say again that it is an honor to welcome you to our institution and that I hope your stay here will be enjoyable as well as profitable.

Frederick C. Cordes, M.D.
Chairman, Department of Ophthalmology
University of California School of Medicine

PRESENT STATUS OF THE VIRAL KERATOCONJUNCTIVITIS PROBLEM*

PHILLIPS THYGESON, M.D.
San Francisco, California

With the effective control of the bacterial forms of keratitis and conjunctivitis by antibiotics and sulfonamides, the viral forms have come into increasing prominence. Trachoma is still the eye disease of greatest world-wide distribution and importance but, although improved therapeutic measures are available, the causal virus has not yet been cultivated with certainty and much work remains to be done on it. Epidemic keratoconjunctivitis has attracted a great deal of popular attention because of the explosive nature of its outbreaks, particularly in factories, hospitals, dispensaries, and medical offices. In the United States, however, the viral keratitis and keratoconjunctivitis of greatest visual and economic importance are due to herpes simplex virus. Herpetic keratitis is now the most important specific type of keratitis and a major cause of impaired vision and blindness. It seems to have become more frequent and severe since the War and there has apparently been an increase in the number of bilateral cases.

In addition to a number of other types of conjunctivitis and keratitis known to be caused by viruses—for example, inclusion conjunctivitis virus, molluscum contagiosum virus, herpes zoster virus, and Newcastle disease virus—there are still a large number of types whose cause is unknown but in which viruses have been suspected because of the absence of bacteria or fungi, and because of suggestive cytologic and pathologic changes.

Following are some brief comments on the various types of conjunctivitis and keratitis known to be, or suspected of being, due to viruses:

* From the Department of Ophthalmology and the Francis I. Proctor Foundation for Research in Ophthalmology, University of California School of Medicine.

TRACHOMA

This chronic keratoconjunctivitis occurs with the greatest frequency in the Middle East where in certain areas over 90 percent of the population is affected. Vision is reduced as a result of the development of corneal infiltrates and vascularization, and finally of scars. Deformity of the lids, due to cicatricial contraction of the tarsi, leads to entropion and trichiasis, and to a more severe keratitis as a result of the trauma caused by the rubbing of the cilia against the cornea. In a disease so chronic that it may remain active during a lifetime, there is inevitably a high incidence of secondary bacterial infection. In the Middle East particularly, superimposed infections with the Koch-Weeks bacillus and the gonococcus create a severe disease, often known as "ophthalmia aegyptiaca," which has a devastating effect on the cornea and on vision.

Although trachoma is now relatively rare in the United States, sporadic cases are still seen in the white population and there has been a recent increase in the incidence of the disease in the Indian population, particularly of the Southwest, and control measures are now being formulated. Because of their infrequency, the sporadic white cases are apt to remain undiagnosed until pannus and cicatrization have developed unmistakably.

The virus has been classified tentatively with the psittacosis-lymphogranuloma group with which it shares a susceptibility to chemotherapeutic agents. The sulfonamides and the broad- and medium-spectrum antibiotics have been remarkably successful in the treatment of individual cases but have been less successful in mass treatment campaigns owing to the prolonged treatment times required.

In spite of intensive study, the virus has so far resisted cultivation in series. This

problem must be resolved and research efforts should also be directed toward (1) improvement in microscopic methods of diagnosis, (2) development of a prophylactic vaccine, and (3) development of a therapeutic agent requiring a minimal treatment time.

Inclusion conjunctivitis

This conjunctivitis, which occurs most frequently in the newborn as one type of ophthalmia neonatorum, is closely related to trachoma but displays none of its corneal or cicatricial complications. Like trachoma, it can be treated successfully with sulfonamides and broad- and medium-spectrum antibiotics, and the treatment time is shorter. The reservoir of the virus is the genitourinary tract where it produces one type of mild, nongonococcic urethritis in the male and a subclinical cervicitis in the female. The widespread use of the sulfonamides and antibiotics for many unrelated diseases probably accounts for the marked reduction in the number of cases of inclusion conjunctivitis seen in recent years.

The disease is of minor economic importance since there is no visual disability connected with it, and since the symptoms, except in the newborn child, are mild. It does have rather extraordinary scientific interest, however, by virtue of its venereal nature and the resemblance the virus bears to the virus of trachoma. Current research is aimed principally at cultivation, but, just as with trachoma virus, all efforts in this direction have so far failed.

Epidemic keratoconjunctivitis

This severe and important keratoconjunctivitis has been recognized as a clinical entity since 1889 but the etiology is still in dispute. All clinical and epidemiologic evidence suggests that the disease is a distinct entity, however. It is widespread in sporadic and epidemic form throughout the world but has the highest prevalence in the Orient.

The disease was not recognized in the United States until 1941 when it was imported from the Far East by way of Hawaii. Since then it has occurred in a number of explosive epidemics in industrial plants, in hospitals, and in medical dispensaries and offices. We have also seen cases in which epidemiologic investigation has failed to reveal contact with known cases, in spite of the fact that the incubation period, usually nine days, favors the gathering of reasonably complete epidemiologic information.

The disease appears typically as an acute keratoconjunctivitis with preauricular adenopathy in which the conjunctival phase precedes by seven to 10 days the onset of the corneal phase. It is the corneal phase that is diagnostic by virtue of the characteristic round subepithelial infiltrates which persist for many months but which eventually disappear, usually without scar formation. It is generally recognized that the disease can occur as a conjunctivitis only. Except in connection with a known epidemic, such cases can then be confused with a number of other forms of acute follicular conjunctivitis.

It can certainly be said that this disease represents a constant threat, since minor epidemics are continually occurring, and major epidemics could occur at any time in our industrial establishments. It was considered a threat to our war effort in 1941 and 1942 when it spread through shipyards and factories. Control measures should be planned for future emergencies.

Research in this disease has been directed principally at establishing etiology and defining epidemiology. Many other problems should also be investigated, however. Among these are (1) the possibility of a vaccine, (2) the perfection of a laboratory means of diagnosis for the detection of cases in the prekeratitis phase, and (3) the development of a trustworthy method for the sterilization of the ophthalmic tonometer, which has been a frequent source of spread. Both clinical and laboratory research are needed for the establishment of criteria for the differential diagnosis of atypical cases from other types

of conjunctivitis and keratoconjunctivitis, particularly from incomplete forms of the pharyngoconjunctival fever syndrome.

The possible etiologic relationship of pharyngoconjunctival fever to Béal's acute follicular conjunctivitis will be discussed later. It is sufficient to say here that the two diseases have much in common, particularly as to clinical course, absence of major corneal complications, transmission in swimming-pools, and the occurrence of epidemics in the fall of the year.

Pharyngoconjunctival fever

This recently described clinical and etiologic entity will be discussed in detail by other participants in the symposium. Unlike epidemic keratoconjunctivitis, this disease has been seen in the United States for many years. The excellent clinical and epidemiologic study of Cockburn in the Greeley epidemic of 1953 brought out the essential features of the disease, particularly its occurrence in greatest numbers in children and the relationship of the swimming-pool to its spread. Here in the San Francisco Bay region important epidemics occurred in 1950 and 1951 in the months of August and September, and in each epidemic swimming-pools were obviously concerned.

One of the major points of discussion in the symposium should be the differential diagnosis of this disease from epidemic keratoconjunctivitis on the one hand, and from Béal's acute follicular conjunctivitis on the other. It is the frequency and character of the corneal lesions that must furnish differentiating data, pending a decision on the etiology of epidemic keratoconjunctivitis and Béal's conjunctivitis.

Newcastle disease conjunctivitis

An acute follicular conjunctivitis, often with preauricular adenopathy, and of short duration (seven to 10 days), is occasionally seen among veterinarians, poultry-men, or workers in packing houses where chickens and other fowls are handled. A number of small epidemics have been reported in this country and abroad. Although the disease in fowls is a pneumo-encephalitis with a definite mortality, the only reported human manifestation of this disease has been the conjunctivitis, which has always been benign, self-limited, and without corneal complications.

The few cases seen by me in the Santa Clara Valley have been in farmers and veterinarians and the conjunctival changes have been indistinguishable from those of Béal's conjunctivis and pharyngoconjunctival fever. The nature of the disease was suspected because the patients had either been in contact with live vaccine or with the disease in fowls, but etiologic diagnosis was possible on a laboratory basis by isolation of the virus or by the demonstration of a rise in antibody during the course of the disease.

This form of viral conjunctivitis is of minor economic importance since it rarely causes any loss of work time and since eye-to-eye spread does not seem to occur. Certainly there have been no visual sequelae. The disease has greater scientific than practical importance, but the development of a simpler means of laboratory diagnosis would be worth further research effort.

Lymphogranuloma venereum keratoconjunctivitis

This serious but rare type of viral keratoconjunctivitis is now amenable to chemotherapy if it is recognized early. It is one cause of oculoglandular conjunctivitis (Parinaud's conjunctivitis) and usually constitutes the initial lesion of the disease. Ocular lesions secondary to the venereal form of the disease have for the most part taken the form of uveitis, scleritis, or interstitial keratitis without conjunctival changes. Most conjunctival cases, at least in this country, have been recognized late except in accidentally infected laboratory workers handling the virus in whom the source of the disease was of course quite obvious.

Diagnosis on the basis of specific con-

junctival or corneal lesions has not been possible, but the development of an elephantiasis of the lids, somewhat suggestive of the elephantiasis of the labia in the female genitourinary disease due to lymph vessel blockage, has been characteristic of late neglected cases. This feature was particularly evident in the case reported by Curth, Curth, and Sanders which I was privileged to see in consultation.

While this form of keratoconjunctivitis has little economic importance in the United States because of its rarity, neglected cases can certainly result in total loss of vision. The investigation of clinical and laboratory methods of early diagnosis is thus very much needed.

Herpetic keratitis and keratoconjunctivitis

This disease of great economic and visual consequence will be the most important subject of this symposium. In spite of the fact that much is now known about the nature and properties of herpes simplex virus, very little progress has been made in methods of clinical and laboratory diagnosis of atypical and early cases. Certainly this disease warrants a major research effort by both ophthalmologists and virologists. Many problems connected with it need elucidation. Some of these will be discussed in this symposium; others we hope at future meetings.

It is of interest that this disease, which in the skin is benign and self-limited, is most serious in the cornea and uveal tract because of its chronicity and tendency to form scars. It should be possible, even in the absence of a specific antiviral therapeutic agent, to protect the cornea against cicatrization, to reduce chronicity, and to prevent or minimize relapses.

Keratitis due to variola and vaccinia viruses

Corneal scars due to smallpox occurred frequently at one time but are now rarely seen. Cases of accidental vaccinial blepharitis and keratoconjunctivitis are fairly common, however, and most ophthalmologists encounter a number of them during their careers. In some cases the lesions are limited to the lids but more commonly involve also the conjunctiva and cornea. The typical corneal lesion is a disciform keratitis which often results in dense cicatrization. The disease is sufficiently common to have both economic and visual importance, and the investigation of control methods would seem to be definitely in order. The modification of the ocular disease by the use of hyperimmune serum should also be investigated further.

Measles keratoconjunctivitis

Conjunctivitis is a regular feature of measles. Koplik's spots are frequently seen on the conjunctiva, particularly on the semilunar fold. Only with the slitlamp and corneal microscope can the characteristic epithelial keratitis be recognized, however. It is this keratitis which in all probability accounts for the common symptom of photophobia. No subepithelial opacities or cicatrical sequelae from the keratitis have been recorded except in a few instances in which secondary bacterial infection has led to corneal ulceration.

In a number of adult cases of measles seen recently, the epithelial keratitis has consisted in multiple epithelial foci, scattered widely over the corneas but most prominent in the pupillary areas. When the foci have been numerous over the pupil, there has been transient reduction in visual acuity. In a few instances these opacities have persisted into the convalescent period.

In view of the transient, benign course of the keratoconjunctivitis of measles, it must be considered of minimal economic and visual importance.

Molluscum contagiosum conjunctivitis and keratoconjunctivitis

When molluscum nodules occur on the lid margins in such a position as to permit virus-

containing epithelial debris to desquamate into the conjunctival sac, a chronic follicular conjunctivitis regularly develops. If the condition persists, the conjunctivitis is frequently complicated by a keratitis, epithelial at onset but sometimes developing subepithelial infiltrates and even pannus. I have seen cases which closely simulated trachoma and in which actual visual loss has occurred. The keratoconjunctivitis does not represent an actual viral infection of the tissues involved but rather a toxic reaction to viral products; extirpation of the lid margin nodules invariably results in rapid cure. It should be mentioned, however, that a few cases have been described in the literature in which molluscum nodules were found on the conjunctiva or cornea; these represented actual virus invasions.

By virtue of its infrequency and the fact that most cases heal without visual loss, molluscum contagiosum keratoconjunctivitis is unimportant economically and visually. Cases are sometimes misdiagnosed, however, particularly when only a single isolated nodule is concerned. The disease is interesting to both ophthalmologists and virologists because the virus has resisted all cultivation attempts, and because the keratoconjunctivitis is unique among viral keratoconjunctivitides (common wart keratitis possibly excepted) in representing a toxic reaction. The resemblance of the keratoconjunctivitis to trachoma has also excited much interest and speculation.

Common wart keratitis and keratoconjunctivitis

Verruca vulgaris of the lid margin is a common finding, but only exceptionally (and in this respect differing from molluscum contagiosum) does a secondary keratitis or conjunctivitis develop as a complication of the lid margin lesion. When a conjunctivitis does develop it is never follicular like molluscum contagiosum conjunctivitis, and the keratitis is strictly epithelial. The keratitis is the more frequent complication and often occurs alone; I have seen some 22 cases of verruca keratitis and most of them have had very little if any associated conjunctivitis. As in molluscum contagiosum, removal of the lid margin nodule results in cure of the conjunctival and corneal disease. Also as in molluscum contagiosum, the viral tumors occasionally occur on the conjunctiva. I have myself seen only one such example.

This disease has considerable theoretic interest but little economic or visual importance. The exact mechanism of the production of the conjunctival and corneal lesions is not understood and further research on this point, and on the purely virologic aspects of the disease, is needed.

Mumps keratitis

Many observers have noted the transient interstitial keratitis which commonly occurs in mumps. Conjunctivitis has not been a feature of the disease. The keratitis has been uniformly self-limited, and, although a drop in acuity has occurred during the illness, recovery has been the rule. In none of the small series of cases I have studied has there been any permanent change; the corneal lesion is presumably an edema without necrosis of stromal cells. A few cases in which the lesion has become disciform, with subsequent scar formation, have been recorded in the literature, however.

The economic importance of mumps keratitis can be considered minimal and visual loss exceptional.

Herpes zoster keratitis

Herpes zoster ophthalmicus is a major eye disease whose frequency has increased in parallel with the increased life span since the majority of cases occur in elderly individuals. During the course of the cutaneous disease, a moderate to severe conjunctival hyperemia commonly develops, but it is the keratitis, often complicated by iridocyclitis and secondary glaucoma, which is most important.

The keratitis is typically subepithelial, without ulceration, and consists in multiple round or oval opacities resembling to some extent the opacities of epidemic keratoconjunctivitis. Many severe cases develop a disciform keratitis, and in my experience the healing of such cases has always been accompanied by extensive cicatrization. Even in the subepithelial opacity cases, a considerable reduction in vision is the rule and is often permanent. There is regularly a reduction in, or complete disappearance of, corneal sensitivity, and as a result trophic changes in the epithelium frequently supervene.

Zoster keratitis is of course diagnosed strictly on a clinical basis. A few atypical cases with minimal skin lesions have been seen in which laboratory aid would have been valuable if it had been available. The disease is important economically and visually and needs a lot of investigation. Gundersen's claim that the corneal phase can be prevented or modified by the use of convalescent blood or serum early in the disease needs confirmation. Little is known of the localization of the virus in the ocular tissues, and indeed many features of the disease need further elucidation.

Varicella keratitis

The eye is only rarely involved in chickenpox. Pox of the lid margins and of the bulbar conjunctiva have been reported, and so has uveitis. A few instances of keratitis, typically disciform, have also been recorded. Although I have seen a number of pox of the lids and conjunctiva, and one case of varicella uveitis, I never have observed the keratitis. Its economic and visual importance must be considered minimal because of its rarity. In view of the apparently close relationship between the varicella and zoster viruses, it is curious that there should be such a difference in the ocular lesions they produce.

Conjunctivitis or keratitis in other known viral diseases

A conjunctivitis, generally consisting in hyperemia, with minimal infiltration and without follicular or significant papillary hypertrophy, is a common feature of most viral diseases with systemic manifestations, for example, yellow fever, German measles, dengue fever, sandfly fever, and the common cold. In a few instances keratitis has been reported but so infrequently as to raise serious doubts that a causal relationship existed in the reported cases. A keratoconjunctivitis of consequence appears to have developed in a few of the rare human cases of foot-and-mouth disease, however. It is of interest that a frequent manifestation of psittacosis in birds is a conjunctivitis in which the specific inclusions are readily demonstrable, but that the disease in man is not accompanied by ocular lesions.

Conjunctivitis and keratoconjunctivitis of suspected viral origin

Béal's acute follicular conjunctivitis

This benign, self-limited, acute follicular conjunctivitis was first defined as a clinical entity by Béal in 1907 when he described an epidemic occurring in Paris. About one-third of his series of cases had systemic symptoms suggestive of pharyngoconjunctival fever, and since the clinical picture and course of the conjunctivitis are similar to those of the latter disease, it is possible that the two are identical. This problem will be discussed later in this symposium.

The disease is characterized by absence of corneal changes, occurrence of a scanty exudate of the typical viral type (mononuclear cells predominating), occurrence in epidemic form, transmission in swimming-pools, and affinity for children and young adults. In the small epidemic reported by me among summer school students at the University of Iowa, all the patients had

frequented swimming-pools and none had any signs of associated systemic disease. It is to be hoped that a decision as to the cause of this disease can soon be reached.

Chronic follicular conjunctivitis (Axenfeld type)

This type of chronic follicular conjunctivitis is characterized by absence of any associated corneal changes, by long duration (average two years) and minimal inflammatory signs, and by a tendency to spread in orphan asylums and other institutions where children are in close contact. Onset is insidious and healing always takes place without sequelae. Nothing is known about etiology but the disease can be transmitted from person to person though not to monkeys or other laboratory animals.

This disease is of no economic or visual importance but is of considerable scientific interest and very much needs investigation.

Superficial punctate keratitis

This distinct clinical entity is characterized by a chronic punctate epithelial keratitis in association with a mild bulbar conjunctivitis. It is typically bilateral and invariably of long duration, but it heals after several years of activity with no sequelae. The disease is a source of considerable discomfort and temporary visual loss. It is of interest that the signs and symptoms can be suppressed almost completely by topical applications of cortisone and other steroids. All age groups are affected.

From this disease Braley has described the isolation of a virus which is specifically neutralized by sera from affected cases. Confirmation of this claim is still lacking and further research is urgently needed. The disease is of considerable economic and visual importance by virtue of its relative frequency, its long duration, the discomfort it engenders, and the temporary visual loss which is a characteristic feature.

Ocular pemphigus

This devastating and usually binocular keratoconjunctivitis is fortunately rare and spares children and young adults. It usually progresses relentlessly to total blindness and is one of the most destructive of all the keratoconjunctivitides. Unfortunate features of the disease are (1) cicatrization, which leads to trichiasis and consequent great discomfort, and (2) loss in tear function which occurs early due to obliteration of the secretory ducts of the lacrimal gland; this in turn leads to cornification of the epithelium of the conjunctiva and cornea.

Nothing is known about etiology. The disease has a great tendency to pick up secondary bacterial invaders, particularly beta hemolytic streptococci, but it seems clear that no bacterium plays a primary role. A characteristic cytologic feature of the disease is the regular occurrence of a low-grade conjunctival eosinophilia and of a blood eosinophilia.

This disease certainly warrants intensive investigation. If it were not for its infrequency it would have great economic importance. There is no doubt about its destructive effect on vision.

Reiter's disease conjunctivitis

The mucopurulent conjunctivitis of Reiter's syndrome has been of signal ophthalmologic interest, although cases are rarely seen in this area. The nonspecific conjunctivitis is usually subacute and associated with a moderate conjunctival exudate in which neutrophils predominate. No significant micro-organisms, inclusions, or other cytologic findings have been noted in conjunctival scrapings and exudates. Only rarely is there any corneal involvement but when it occurs it is usually in the form of marginal infiltrates.

This disease, whose cause is still unknown in spite of isolation claims for both viral

and pleuropneumonialike organisms, is of importance because it attacks young male adults of military age predominantly, and because the associated arthritis is incapacitating over periods of several months.

Conjunctivitis of the erythema multiforme syndrome

The conjunctivitis of this syndrome may be catarrhal, purulent, or membranous. The purulent and membranous forms may lead to important cicatricial changes of the conjunctiva and cornea, with diminution or loss of vision. The etiology is still unknown. Since the disease occurs frequently and attacks children and young adults predominantly, often effecting serious visual loss, it is of distinct economic importance. Further research is clearly indicated.

Other types of keratoconjunctivitis of indefinite nature

During the course of our keratoconjunctivitis studies here at the University of California since 1946, we have encountered a number of cases of keratoconjunctivitis which have defied clinical or laboratory analysis. Some of them have been mild and self-limited, but a significant number have been chronic, severe, and accompanied by major visual loss. These cases were studied intensively for etiologic agents without results. It is obvious that the study of this type of case warrants a major effort in virus research.

University of California
Medical Center (22).

CLINICAL MANIFESTATIONS OF PHARYNGOCONJUNCTIVAL FEVER*

JOSEPH A. BELL, M.D.
Bethesda, Maryland

This discussion of the clinical manifestations of pharyngoconjunctival fever is limited to the manifestations observed in some 500 cases studied in the Washington, D.C., area,[1-4] because other speakers will describe the disease as seen elsewhere. The observers included Dr. R. J. Huebner, Dr. W. P. Rowe, Dr. R. G. Suskind, Dr. R. H. Parrott, Dr. R. W. Ryan, Dr. J. I. Engler, and Dr. J. A. Bell. Epidemiologically, the disease has occurred in all age groups, but predominantly in children; it has occurred in outbreaks in a children's day camp, in an orphanage, and in two residential neighborhoods. It has occurred endemically or sporadically throughout all seasons. Our naturally occurring cases have been caused by Type 3 adenovirus but we have been able to induce a similar illness in many susceptible volunteers by swabbing the conjunctiva with live adenovirus Types 1, 3, 4, and 5, and perhaps 7.[5,6]

Pharyngoconjunctival fever is an acute infectious illness typically characterized by fever, pharyngitis, and a nonpurulent follicular conjunctivitis. These manifestations occured singly or in any combination and with a wide range in degree of severity. Typical cases having the symptom triad were readily curred singly or in any combination and with conjunctivitis served to differentiate the disease from the great mass of undifferentiated acute respiratory illnesses. The conjunctivitis did not occur in some 30 percent of the cases but these could be recognized epidemiologically with a fair degree of accuracy through their association with typical cases. The diagnosis may be confirmed in the laboratory by isolation of the etiologic agent in tissue culture and by a specific antibody rise in acute and convalescent serums.

The onset was either gradual or sudden. The fever rose rather rapidly to as high as 103°F. or 104°F., particularly in children, and gradually subsided by lysis. It persisted from one to 10 days with a median of about five days. Chart 1 shows the febrile course of a typical case in a six-year-old girl seen at the Clinical Center and reported in Dr. Parrott's paper.[1] In this case, the total and differential leukocyte count were not remarkable but in general a slight leukopenia may exist. The pulse and respiration rates followed the temperature curve and were otherwise not unusual.

The pharyngitis was commonly manifest by a sore throat complaint. This was described more as a discomfort or scratchiness rather than painful deglutition. Examination of the throat generally showed nothing striking except that the posterior oral pharynx was frequently studded with glairy lymph follicles associated with vascular injection. Nontender, submaxillary lymphadenopathy was common even in the absence of the sore throat complaint.

The conjunctivitis was occasionally the source of the chief complaint. The eye symptoms ranged from itching and burning to a moderately severe irritation and foreign body sensation. Visual blurring occasionally occurred with excessive lacrimation. Complaints of retro-orbital pain and photophobia were almost nonexistent. Occasionally only one eye was involved, sometimes both eyes were involved simultaneously, and not infrequently the second eye was involved a few days after the first and generally to a milder degree.

* From the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

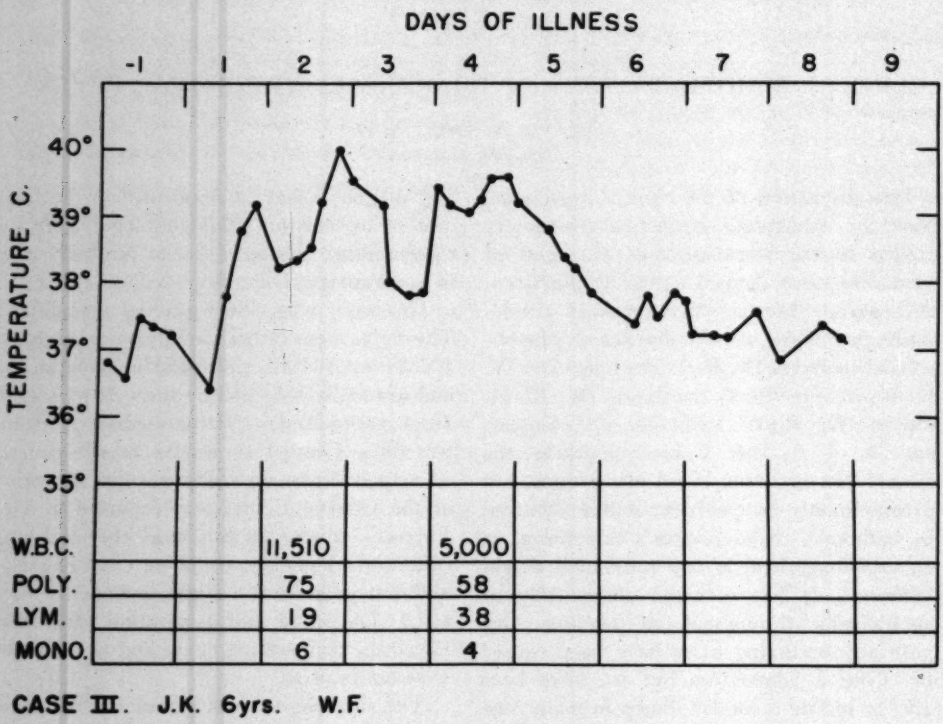

Chart 1 (Bell). Febrile course of a typical case in a six-year-old girl. (Reproduced from Parrott[1] with permission of the publishers.)

Upon examination some narrowing of the palpebral fissure was frequently noted and was considered to be caused by blepharospasm. Often there was some dried serous exudate at the base of the lashes. A purulent exudate was seldom seen. An acute catarrhal inflammation occurred on the lower palpebral conjunctiva and commonly was severe enough to extend to the bulbar and upper palpebral conjunctiva. Lymph follicle hyperplasia was usually present and commonly most marked on the lower palpebral conjunctiva. No involvement of the cornea or any other part of the eye was noted. The preauricular lymph node was occasionally palpable. The conjunctivitis persisted from three to four days to as long as three weeks and recovery was complete. The conjunctivitis was generally less severe in cases occurring during the winter time when not associated with swimming. In two outbreaks occurring in January and April in a Washington, D.C., orphanage, the conjunctivitis was definitely more mild and lasted only a few days to a week.

No deaths are known to have occurred, and no sequelae have been recognized. Otitis media may be a complication or it may be part of the disease. It occurred infrequently and was usually nonpurulent and did not seem to respond well to antibiotics. Headache was a common symptom, and listlessness, particularly drowsiness, toward the end of the febrile illness seemed to be quite common. Cough, chest findings, and skin rashes were practically nonexistent. Nausea, vomiting, diarrhea, and epistaxis occurred only in a few cases. Jaundice did not occur. Muscle, bone, and joint aches and weakness occasionally occurred, particularly in adults.

Differential diagnosis should consider leptospirosis, influenza, herpangina, and various nonpurulent conjunctivitides, for example, herpes, inclusion conjunctivitis, epidemic keratoconjunctivitis. The clinical picture is not that of leptospirosis, as the patients studied did not have the severe malaise, headache, muscle, bone, and joint ache, vomiting, and stiff neck and did not have the jaundice often seen in cases of leptospirosis. In addition, the conjunctivitis in the patients studied in the summer outbreaks was generally of longer duration, and no deaths occurred. Agglutination-lysis tests for leptospirosis aid differential diagnosis particularly in epidemics associated with swimming pools.

The rather sharp outbreaks of acute febrile illness suggest influenza. The patients studied did not generally have the muscle, bone, joint, and retroorbital pains and aches common with influenza; on the other hand, they commonly had a follicular conjunctivitis, which is definitely not common in influenza. The hemagglutination inhibition test and influenza virus isolation aid differential diagnosis, particularly in wintertime outbreaks.

Herpangina is a common febrile disease of children that occurs during the summer months. Clinically, the characteristic herpetiform lesions of herpangina on the palate and anterior pillars serve for diagnosis. The laboratory isolation of Coxsackie virus aids differential diagnosis.

Of the nonpurulent conjunctivitides, epidemic keratoconjunctivitis and inclusion conjunctivitis are to be considered in differential diagnosis. Neither of these is commonly associated with general symptoms nor has such short duration as found in the study cases. In addition, no corneal involvement and no inclusion bodies were found in our cases.

Pathologically, the adenoviruses tended to localize in the lymphatic tissues. When live adenovirus was swabbed on one eye of susceptible volunteers, conjunctivitis developed in two to seven days and not uncommonly the preauricular lymph node, the tonsil, and submaxillary lymph glands became involved, notably on the same side as the affected eye. Cytologic examination of conjunctival smears showed nothing abnormal and no cytoplasmic or nuclear inclusions. Histopathologic examination of conjunctival sections showed that the conjunctivitis was characterized predominantly by migration of lymphocytes into the submucosal tissue. Sections of the tonsil showed that the clinical enlargement was due to hypertrophy and hyperplasia of the tonsillar lymphoid tissue augmented to a slight extent by congestion of vessels and edema of the peritonsillar connective tissue.

We have not carried out any studies on treatment but many of the cases we saw had been treated with the common antibiotics and sulfonamides and no particular benefit was discerned. It appears that topical application of the corticosteroids may alleviate symptoms but may be dangerous if the case is incorrectly diagnosed. Vaccine prophylaxis appears to be effective.[7-9]

National Institutes of Health (14).

References

1. Parrott, R. H., Rowe, W. P., Huebner, R. J., Bernton, H. W., and McCullough, N. M.: Outbreak of febrile pharyngitis and conjunctivitis associated with type 3 adenoidal-pharyngeal-conjunctival virus infection. New England J. Med., **251**:1087-1090 (Dec.) 1954.

2. Bell, J. A., Rowe, W. P., Engler, J. I., Parrott, R. H., and Huebner, R. J.: Pharyngoconjunctival fever: Epidemiologic studies of a recently recognized disease entity. J.A.M.A., **157**:1083-1092 (Mar.) 1955.

3. Ryan, R. W., O'Rourke, J. F., and Iser, G.: Conjunctivitis in adenoidal-pharyngeal-conjunctival virus infection, pharyngoconjunctival fever: A newly described entity. Arch. Ophth., **54**:211-216 (Aug.) 1955.

4. Unpublished data.

5. Bell, J. A., Ward, T. G., Huebner, R. J., Rowe, W. P., Suskind, R. G., and Paffenbarger, R. S., Jr.: Studies of adenoviruses (APC) in volunteers. Am. J. Pub. Health, **46**:1130-1146 (Sept.) 1956.

6. Ward, T. G., Huebner, R. J., Rowe, W. P., Ryan, R. W., and Bell, J. A.: Production of pharyngoconjunctival fever in human volunteers inoculated with APC virus. Science, **122**:1086-1087, 1955.

7. Huebner, R. J., Bell, J. A., Rowe, W. P., Ward, T. G., Suskind, R. G., Hartley, J. W., and Paffenbarger, R. S., Jr.: Studies of adenoidal-pharyngeal-conjunctival vaccines in volunteers. J.A.M.A., **159**: 986-989 (Nov.) 1955.

8. Hilleman, M. R., Stallones, R. A., Gauld, R. L., Warfield, M. S., and Anderson, S. A.: Prevention of acute respiratory illness in recruits by adenovirus (RI-APC-ARD) vaccine. Proc. Soc. Exper. Biol. & Med., **92**:377-383 (June) 1956.

9. Bell, J. A., Hantover, M. J., Huebner, R. J., Loosli, C. G.: Efficacy of trivalent adenovirus (APC) vaccine in naval recruits. J.A.M.A., **161**:1521-1525 (Aug.) 1956.

SPORADIC CASES OF PHARYNGOCONJUNCTIVAL FEVER*
In Northern California, 1955-1956

S. J. Kimura, M.D., L. Hanna, M.A., A. Nicholas, B.A., P. Thygeson, M.D., and E. Jawetz, M.D.
San Francisco, California

During the past 15 months we have studied 27 patients with acute conjunctivitis associated with symptoms of upper respiratory infection with pharyngitis, preauricular and/or cervical adenopathy, and fever. The patients were all from the San Francisco Bay area, and many of them were referred by local ophthalmologists, the Eye Clinic, and Student Health Service of the University of California Medical Center of San Francisco.

Methods

Criteria for including patients in series

All the patients included in this series were examined thoroughly with a slitlamp microscope, and those with acute follicular hypertrophy of the conjunctiva were included. Bacterial cultures were taken to rule out bacterial infection, and cytologic study of conjunctival exudate was made to show the predominantly mononuclear cell reaction which is characteristic of viral infections. Patients were questioned as to the presence of respiratory symptoms. Routine throat examinations were made to determine if there were a pharyngitis present.

* From the Departments of Ophthalmology and Microbiology and the Francis I. Proctor Foundation for Research in Ophthalmology, University of California School of Medicine.

Virus isolations

Washings or scrapings from conjunctiva were obtained. Throat swabs were obtained from patients exhibiting respiratory symptoms. The specimens were suspended in maintenance medium (10-percent chick serum in Mixture 199), with the addition of antibiotics to prevent bacterial growth and were either inoculated into tissue culture immediately or kept frozen at −20°C. until used. HeLa cell tissue cultures in tubes were obtained from Tuskegee Institute, Alabama. They were washed twice with Hanks' solution, and then 0.2 ml. of the specimen and 0.8 ml. of maintenance medium were added.* The tubes were incubated in a stationary position at 36°C. for as long as the integrity of the HeLa cells permitted, usually 12 to 21 days, with occasional changes of maintenance medium when required by acid pH. The tubes were observed regularly at intervals of one or two days for cytopathogenic effects indicative of virus proliferation. When no evidence of virus activity was detected in the first tissue culture passage, at least one blind passage was made before the specimen was discarded as negative.

Viruses isolated in tissue culture were

* All tissue culture media were obtained from Microbiological Associates, Bethesda, Maryland.

typed with known specific antisera through the assistance of Lieut. Col. T. O. Berge, 6th Army Area Medical Laboratory, and Dr. R. J. Huebner, National Institute of Health.

Serologic tests

Complement-fixation tests were performed in a conventional manner, using two full units of complement, two units of hemolysin, two units of antigen, and a two-percent suspension of sheep cells. Sera were inactivated at 60°C. for 20 minutes. The test was incubated over night at 4°C. and for 10 minutes at 37°C. before the addition of sensitized cells. Readings were taken 10 minutes after the controls cleared completely. The antigen consisted of fluid from tissue cultures in which types 3, 4, 6, or 8 adenoviruses had been grown. Immediately before use this fluid was heated at 56°C. for 30 minutes, then centrifuged at 1,000 rpm for 10 minutes.

For neutralization tests about 100 $T.C._{50}$ doses of infective virus were mixed with twofold serum dilutions and incubated at room temperature for one hour. The 0.2 ml. of the mixture was inoculated into each tube of twice-washed HeLa cell cultures, and 0.8 ml. of maintenance medium was added. The tubes were incubated at 36°C. in a stationary position and read daily in the conventional manner, denoting cytopathogenic effects by 1+ to 4+. Agreement between duplicate tubes containing the same mixture was good. The result was considered to show neutralization when there was a difference of 3+ in readings of experimental and control tubes for at least two consecutive days. Virus titrations and positive and negative control sera were included in each test.

Analysis of cases

Age of patients

The patients' ages ranged from two to 41 years, with a median age of 25.5 years. The majority were young adults, with 21 of the 27 patients ranging in age from 15 to 30 years. Four of our patients were resident physicians and two were student nurses, two pharmacy students, and one dental student. None of these patients gave a history of exposure to patients with conjunctivitis or to swimming.

Clinical symptoms

The outstanding symptom was conjunctivitis in all of the 27 patients. The conjunctivitis was acute in most cases with follicular hypertrophy of the conjunctiva. The exudate was never purulent but always serous. Bacterial cultures were uniformly negative for pathogenic organisms, and the cytology of the exudate showed predominantly mononuclear cell reaction.

Four of the 27 patients had a definite epithelial keratitis along with the follicular conjunctivitis. The earliest case seen was three days after onset, and the keratitis was marked. Only a few of these lesions stained with fluorescein after a week or 10 days. By biomicroscopic examination the early lesions appeared as groups of small opacities, forming small round, linear, or stellate opacities which were totally confined to the corneal epithelium. These lesions cleared gradually, leaving a group of larger opacities more sparsely distributed. In one case these lesions have lasted four weeks with gradual fading.

There were other associated signs and symptoms present in all of the patients. Preauricular adenopathy on the same side as the eye lesion was present in 23 of the 27 patients (85 percent). Pharyngitis was present in 15 of the patients. Only a few of the patients were aware of having a fever, and when checked with a thermometer only nine of 27 patients had a definite fever.

Signs and Symptoms

	Patients
Conjunctivitis, follicular	27
Adenopathy, preauricular	23
Pharyngitis	15
Fever	9
Upper respiratory infection	8
Keratitis	4
Adenopathy, cervical	3
Malaise	3

Virus isolations

Adenovirus was cultured from 13 of the 27 patients. Adenovirus type 3 was isolated from 11 of the patients, type 2 from one patient, and type 6 from another. All of these cases were cultured within eight days from the time of onset of the conjunctivitis. Nine of the 13 positive cultures were obtained by washings of the conjunctival sac. In two patients the conjunctival scrapings were taken as well as conjunctival washings. Scrapings alone were taken on one case. Positive throat cultures were found in four patients in whom virus was cultured from the conjunctival sac and in each instance the virus type was the same.

Serologic studies

Paired sera were available from only 10 of the 13 patients who yielded adenoviruses. Ten of these patients had a significant and diagnostic rise in specific antibodies. Neutralizing antibodies showed a significant rise in all 10 patients, but complement fixing antibodies rose in only seven of them. In the remaining 14 patients from whom we failed to recover adenoviruses serologic tests have not been completed.

Discussion

Twenty-seven cases of acute follicular conjunctivitis with systemic or respiratory involvement, but without seasonal incidence, were studied. All of these cases were diagnosed clinically as pharyngoconjunctival fever, even though the systemic and respiratory symptoms were often mild or even absent. Preauricular and/or cervical adenopathy was the only fairly constant finding along with the follicular conjunctivitis. This is unlike the reports of Bell et al., and Ryan et al., of the disease in children where symptoms such as fever, malaise, pharyngitis, and headaches were more prominent than the conjunctivitis. Most of the patients studied in this report were adults whose primary complaint was an acute red eye.

An adenovirus was isolated from 13 of the 27 cases. All were type 3 adenovirus except in two patients from whom a type 2,* and a type 6,† respectively, were isolated. In both of these cases there was a corresponding rise in neutralization titer against the virus isolated.

The keratitis was present in only four of the 27 patients. During the early stage of the disease the keratitis can be missed unless specifically looked for with biomicroscope. The keratitis is strictly epithelial and the lesions are small and diffusely scattered over the whole cornea. The larger opacities in the later stage are still epithelial, but the opacity may involve the subepithelial part of the cornea also. These are easily differentiated from the opacities of epidemic keratoconjunctivitis because the main part of the opacity is in the epithelium and often stains with fluorescein. Very often the opacity causes a thickening of the epithelium which can be seen by reflected light. Unlike epidemic keratoconjunctivitis these opacities are not necessarily grouped in the area of the papillary axis, but scattered over the whole cornea. Also the opacities fade sooner than those of epidemic keratoconjunctivitis which may last up to three years.

Sporadic cases of pharyngoconjunctival fever in Northern California occur primarily in young adults and the systemic and respiratory symptoms appear to be less severe than in children. Both type 2 and 6 adenovirus appear to be an etiologic cause of pharyngoconjunctival fever in addition to the type 3.

University of California
Medical Center (22).

---

* Jawetz, E., Hanna, L., Kimura, S. J., and Thygeson, P.: Clinical diseases of adults associated with sporadic infections by APC viruses. Arch. Int. Med., **98**:71-79, 1956

† Jawetz, E., Hanna, L., Kimura, S. J., and Thygeson, P.: A new type of APC virus from follicular conjunctivitis. Am. J. Ophth., **41**:231-234, 1956.

CANADIAN CASES OF ADENOVIRUS INFECTION 1951-1956*

H. L. Ormsby, M.D., Ann M. C. Fowle, Ph.D., and Frances Doane, B.Sc.
Toronto, Ontario

In 1950, a study of eye virus infections at the University of Toronto was commenced with the aid of a National Health Grant. Since its initiation, this work has gone on continuously, and much information has been accumulated concerning eye infections in the Toronto area. All residents in the Department of Ophthalmology have had a period of instruction in the Eye Bacteriology and Virus Laboratory during the early months of their training. In their subsequent years in the eye clinics they were thus able to recognize eye virus diseases and referred many of the patients in this study to the laboratory for further work-up. Thus, a fairly accurate knowledge of the epidemiology of the adenovirus diseases affecting the eyes is presently available in the areas serviced by the teaching hospitals in metropolitan Toronto.

Our initial interest in the group of eye virus diseases, now known as adenoviruses, was stimulated by an extensive outbreak of epidemic keratoconjunctivitis which occurred in 1951 at the Ford Motor plant in Windsor, Ontario.[1] Epidemiologically and clinically this outbreak presented many interesting features. The early cases, occurring in the spring of 1951, were relatively benign and few patients developed corneal opacities. At the height of the epidemic, in June and July, the course of the disease was more acute, and many patients developed keratitis. The severity of new cases occurring in the last few weeks of July decreased, and the disease disappeared entirely during August when the plant was closed down for holidays. There was no evidence that epidemic keratoconjunctivitis was epidemic elsewhere in the city of Windsor, and oculists who were practicing in that area saw few cases originating outside the Ford Motor plant. The transmission of this disease in this epidemic was similar to that reported in previous outbreaks throughout the United States during the past 10 years. Very largely the disease was confined to industries and spread within the plants without significantly affecting persons working in other industries or in the population at large.

Clinically, this epidemic of epidemic keratoconjunctivitis was interesting since out of over 600 patients who presented themselves for treatment, only 89 developed corneal opacities seen by focal illumination and the binocular loupe. The corneal opacities were typical of those described in previous epidemics of epidemic keratoconjunctivitis, being dense, round, sharply circumscribed epithelial and subepithelial infiltrates located chiefly about the visual axis.

In a follow-up study of those patients with corneal opacities one year later, 29 of the 61 patients available for study still had corneal opacities with resultant visual disturbance. A number of nonopacity cases were examined by one of us (H. L. O.) and were seen to have a similar course to those with opacities, in that there was a sharp onset with a foreign-body sensation, tearing, and injection of the conjunctiva. There was usually an enlargement and tenderness of the preauricular node on the affected side. Follicles appeared in the conjunctiva of both fornices as the disease progressed but were not as large as those seen in inclusion conjunctivitis. Pseudomembranes and subconjunctival hemorrhages were frequently present. The second eye was involved within two to five days in most instances, but the course was invariably more benign.

In Toronto, at the time of the Windsor epidemic, a number of sporadic cases of follicular conjunctivitis were seen by us, and

* From the Departments of Ophthalmology and Bacteriology, Faculty of Medicine, University of Toronto, and the Research Institute, The Hospital for Sick Children, Toronto.

carefully followed by slitlamp microscopy. These cases were similar to the nonopacity cases seen in Windsor. Only one of these patients (a farmer) developed typical major subepithelial opacities.

During the next two years (1952-53) attempts were made to isolate virus from frozen stored washings taken from these Windsor and Toronto cases during the acute phase of the disease, using tissue cultures of embryo mouse-brain according to the technique previously reported by Sanders and Alexander. A number of strains of virus were isolated, but these were all subsequently shown to be closely related to the Theiler T. O. strain of mouse encephalomyelitis virus. Sixty-one convalescent sera from opacity cases in Windsor were then tested against the Braley-Sanders virus. None of them was found to have neutralizing antibody to this virus. Similarly, none of the convalescent sera from the Toronto cases had antibodies to this virus.[2]

In the Toronto area, during the five years of this study, only four patients with the typical major opacities of epidemic keratoconjunctivitis have been seen by us. Two of these showed a rise in neutralizing antibody to the type 8 adenovirus (Trimborn). Two other patients who had corneal opacities indistinguishable from those of epidemic keratoconjunctivitis, immediately following the acute phase of the disease, failed to show a rise of antibody to the Trimborn virus. In one of these patients, the type 3 virus was isolated, and the opacities did not persist beyond a six-month period. In the other patient, the opacities faded and had disappeared within four months.

During 1952, 1953, and the first 10 months of 1954, no epidemics of eye virus infection were seen by us in the Toronto area. One patient was seen in August, 1954, with a follicular conjunctivitis, preauricular adenopathy, and subconjunctival hemorrhages. In November, 1954, another patient was seen with the same symptoms, and from this time onward throughout 1955 patients with follicular conjunctivitis came to us in increasing numbers until the disease reached epidemic proportions.

Meanwhile Rowe and co-workers[3] had reported the isolation of a new group of viruses which they named the "APC group." Since monkey-kidney and HeLa cells were now available to us, attempts were made to isolate virus in tissue cultures with these new cells from eyewashings and throat swabs. At the time of this report, a total of 42 virus isolations have been completed, and typing has revealed that 23 were neutralized by the type 3 adenovirus antiserum, 15 by type 7, one by type 2, one by type 9, and one strain is not yet typed. The reports of these isolations are available in separate publications.[4,5] In addition to the patients from whom isolations of virus have been obtained, all of whom were examined by us, several hundred additional patients were seen by one of us (H. L. O.) with clinical manifestations of the disease, usually in the late stages of the disease so that eyewashings were not taken. Thus, during 1955, the epidemic of pharyngoconjunctival fever was widespread and of serious proportions.

In an analysis of patients from whom virus was isolated, it will be seen in Table 1 that type 3 infections occurred throughout 1955, with the highest incidence in the summer months. A sharp epidemic of type 7 adenovirus infection, transmitted largely in swimming pools, appeared later in July and lasted only for five weeks.

The signs and symptoms of type 3 and type 7 adenovirus infection in these epidemics were similar in most respects. The majority of the patients from whom type 3 virus was isolated were adults, none of whom had significant systemic manifestations, but all of whom were seriously enough concerned about their ocular manifestations to seek medical aid. We were able to follow most of these patients with slitlamp microscopy over periods sufficiently long to reveal the presence or absence of corneal opacities. In the corneas of 24 patients from whom

TABLE 1

DATE OF ONSET OF PHARYNGOCONJUNCTIVAL FEVER, PROVEN BY VIRUS ISOLATION

	Number of Cases				
	Type				Unknown
	2	3	7	9	
1954					
December		2			
1955					
January					
February					
March		2			
April					
May		5			
June					
July		1	2		
August	1	7	11	1	
September		1	2		
October		2			
November		1			
December		2			
1956					
January		1			
August					1
Total:	1	24	15	1	1

type 3 virus was isolated, corneal opacities were noted in 10, or approximately 50 percent. In neither of the two children in the type 3 group, aged nine years, were corneal changes observed. All patients with opacities were adults. Similarly, in two of the four adult patients from whom type 7 adenovirus was isolated, corneal opacities were seen on slitlamp microscopy. In none of the children with type 7 virus were any corneal changes observed.

When keratitis developed in the affected eyes, it was seen to follow a characteristic course. Usually in the first eye developing the disease, and hence the most acutely involved, epithelial erosions, staining with fluorescein, appeared between the eighth and 10th days after onset. At this time, the patient usually complained of increasing photophobia. Some patients dated the onset of their disease from the time of onset of the keratitis, dismissing the initial tearing and redness as being negligible. Forty-eight to 72 hours after the onset of the staining areas, nebulous infiltrates could be detected in the stroma deep to the erosions. These infiltrates increased in density over the next few weeks while the erosions disappeared. In about 50 percent of the opacity cases, where epithelial erosions were numerous, the stromal reaction was severe, and there was diffuse stromal edema with reduction of vision to 20/40ths or 20/60ths. This stromal edema never persisted for more than three weeks. The opacities in two instances were initially confused with those of epidemic keratoconjunctivitis, but in one of these they had resolved by the end of four months, and in the other in six months.

DISCUSSION

No particular significance is attached to the fact that most of our patients from whom type 3 adenovirus was isolated were adults (21 out of 24) or that most of the type 7 patients were children (11 out of 15). In the epidemic due to type 7 adenovirus many children were admitted to hospital and were available for study. Histories taken on individual cases in both type 3 and type 7 infections showed that the disease was transmitted readily in the home, both to the adults and to children. The high percentage of corneal opacities in adults seen by us (50 percent) is probably due to the fact that only those adults with relatively severe symptoms sought medical advice, and came to us in most instances by referral from public clinics and private offices.

Our experiences with adenovirus infections are similar to those of others reported in the past few years from other sections of the continent. Epidemics of eye virus infection of this type were unknown or previously unreported in Canada. They do resemble, however, the reports on Béal's conjunctivitis which has been seen sporadically in the United States and in epidemic form in Europe during the past century. The high incidence of the disease during the summer months is probably related to swimming pool transmission which has been previously noted in both the Greeley epidemics in Colo-

rado in 1951[6] and in the Baltimore and Washington epidemics in 1954.[7]

The occurrence of corneal opacities in both type 3 and type 7 adenovirus infections is of major interest to ophthalmologists. Visual impairment in an eye, lasting up to three months, had not been reported in the literature in connection with types 3 and 7 adenovirus infections until the publication of these studies.[4] Further investigations of a similar nature will be necessary to determine if other types of adenoviruses, other than types 3, 7, and 8 can also affect the cornea.

The isolation of type 9 adenovirus from the eye of a patient with follicular conjunctivitis has not previously been reported in the literature.

Banting Institute (5).

REFERENCES

1. McGavin, M. F., Boley, J. P., and Ormsby, H. L.: Epidemic keratoconjunctivitis. Canad. M.A.J., **69**:137-140, 1953.
2. Ormsby, H. L., and Fowle, A. M. C.: Epidemic keratoconjunctivitis. Am. J. Ophth., **38**:490-495, 1954.
3. Rowe, W. P., Huebner, R. J., Gilmore, L. K., Parrott, R. H., and Ward, T. G.: Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exper. Biol. & Med., **84**:570-573, 1953.
4. Fowle, A. M. C., Cockeram, A. M., and Ormsby, H. L.: Virus isolations from patients with keratoconjunctivitis. Am. J. Ophth., **40**:180-188 (Nov. Pt. II) 1955.
5. Beale, A. J., Doane, F., and Ormsby, H. L.: Studies on adenovirus infections of the eye in Toronto. Keratoconjunctivitis symposium, 1956.
6. Cockburn, T. A.: An epidemic of conjunctivitis in Colorado associated with pharyngitis, muscle pain, and pyrexia. Am. J. Ophth., **36**:1934-9, 1953.
7. Bell, J. A., Rowe, W. P., Engler, J. I., Parrott, R. H., and Huebner, R. J.: Pharyngoconjunctival fever. J.A.M.A., **157**:1083-1092, 1955.

ADENOVIRUSES AS ETIOLOGIC AGENTS IN CONJUNCTIVITIS AND KERATOCONJUNCTIVITIS*

ROBERT J. HUEBNER, M.D., AND WALLACE P. ROWE, M.D.
Bethesda, Maryland

The adenoviruses comprise a family of prevalent agents which occur chiefly in man and some of the higher primates.[1-4] In man, they produce catarrhal inflammation of the mucous membranes of the respiratory and ocular systems, accompanied by follicular enlargement of the submucous lymphoid tissues and regional lymph nodes. There are at least 18 serologically distinct human serotypes, 17 having so far been given numbered designations. The adenoviruses have similar physical, chemical, and biologic properties and share a common soluble complement-fixing antigen. The virus particles, as measured in tissue culture fluids, apparently measure 80 to 120 mmμ in diameter, whereas the particles observed intracellularly in the nuclei, as crystal-like patterns, measure from 50 to 65 mmμ. The viruses are ether resistant, highly stable, and, to the extent to which they have been tested in laboratory animals, fail to produce disease in such hosts. In tissue culture, the human agents prefer to grow in epithelial cells, particularly those from the human species. They also grow in human fibroblasts, but at much slower rates; they have been adapted to grow in other tissues such as monkey-kidney tissue cultures. The

* From the Department of Health, Education, and Welfare, U.S. Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Infectious Diseases.

adenoviruses contain, for the most part, potent antigens which, following an infection or their use as vaccines, result in satisfactory complement-fixing, neutralizing, and protective antibodies.

The inclusion of a newly recognized virus type within the adenovirus group is dependent primarily on the demonstration of the common group complement-fixing antigen and the biologic attributes characteristic of the prototypes now recognized.

Adenoviruses represent some of the most common and widespread of human viral parasites. Although studied most extensively in North America, the agents have been demonstrated wherever looked for in various parts of Western Europe, Russia, Czechoslovakia, Arabia, and Japan. Complement-fixing antibodies to this group of viruses are prevalent in surveys not only in the human species, but also in chimpanzees, monkeys, and guinea pigs. However, the high prevalence of many of these agents in man is more accurately reflected in surveys for specific neutralizing antibodies to different serotypes in human serums.

Importance of adenoviruses in producing human disease

Present evidence has established type 3 as a cause of pharyngoconjunctival fever, as well as of acute respiratory disease, febrile pharyngitis, and a certain proportion of simple follicular conjunctivitis. Although more evidence is required, present data are sufficient to suggest that types 1, 2, 5, and perhaps others, are also responsible for common febrile pharyngitis in general population groups, particularly in young children.

It is apparent from the literature that the lion's share of attention has been given to the role of adenoviruses in respiratory illness. Types 3, 4, and 7, causing acute respiratory disease in military recruits, have received widespread attention as a military and public health problem. Indeed, vaccines have already been developed and have been shown to be sufficiently effective so that it is now possible to predict that commercially produced vaccines will soon be in general use in those population groups known to suffer high rates of respiratory disease due to these agents. But it is obvious that the respiratory diseases known to be caused by several of these viruses as they occur in military recruits, a captive population easily observed, represent only a small if concentrated proportion of actual human experiences with these agents.

Serologic surveys and surveys for masked agents in adenoids and tonsils show that the majority of children have been infected with at least two adenovirus types (usually types 1 and 2) prior to entering grade school. Antibodies versus types 3, 4, 5, and 7 are found to occur in the majority of adult serums. On the other hand, antibodies to types 8, 9, and 10, and to the chimpanzee and some of the monkey strains, are comparatively rare in contemporary and limited surveys of human serum specimens.

Importance and occurrence of adenoviruses in conjunctivitis and keratoconjunctivitis

Despite the attention given to adenoviruses as a cause of respiratory disease, their role in the etiology of ocular diseases may well turn out to be equally if not more important. Certainly, the recent observations reported in this symposium guarantee that their occurrence in ocular diseases will be at least equally interesting.

The events which first suggested that adenoviruses (initially called AD agents, and later referred to as RI, APC, and ARD viruses) cause conjunctival disease were accidental infections with type 3 in laboratory and clinical investigators at the National Institutes of Health, three of whom developed unilateral catarrhal and follicular conjunctivitis during the autumn of 1953. Alerted, as a consequence, to look for an illness with conjunctivitis, we observed several outbreaks due to adenovirus type 3 in the succeeding several months.

The first outbreak, studied in detail and reported by Dr. Robert Parrott of the National Institutes of Health, involved patients, nurses, and physicians on the Infectious Disease Service of the NIH Clinical Center.[5] Shortly after this, an extensive outbreak, described in more detail in this symposium by our associate, Dr. Joseph Bell, occurred in a summer day-camp in northern Virginia.[6]

A triad of major clinical manifestations, namely, fever, pharyngitis, and conjunctivitis, led to the suggestion that this highly specific and distinct clinical entity be named pharyngoconjunctival fever. It now appears, from a review of the literature, that this disease was recognized as a distinct entity on a number of occasions previously, perhaps first by Bèal.[7] It is quite apparent, from Dr. Tanaka's translations of the Japanese literature,[8] that an illness similar to pharyngoconjunctival fever was recognized as prevalent in Japanese infants before 1944. This so-called "pseudomembranous conjunctivitis" with systemic symptoms was believed to be a childhood manifestation of epidemic keratoconjunctivitis. It seems likely that the transmission by Japanese workers of what was thought to be the keratoconjunctivitis virus, to human volunteers, actually represented, in some instances at least, a transmission of viruses capable of causing chiefly pharyngoconjunctival fever.

It is also very likely that certain illnesses described by Derrick in 1943 as "swimming bath" conjunctivitis,[9] and an extensive Colorado outbreak of illness studied in 1951 by Cockburn,[10,11] which he called "Greeley disease," represented adenovirus infections.

It is too early, of course, to assess the total significance of the adenoviruses in ocular disease. However, reports on the association of local adenovirus infections with conjunctival and corneal lesions reveal highly convincing evidence of the etiologic roles of certain serotypes in pharyngoconjunctival fever and epidemic keratoconjunctivitis. On the basis of published work, we conclude that serotype 3 is a well-established cause of pharyngoconjunctival fever. Evidence of high order indicates that type 7, or serologic variants thereof, also cause pharyngoconjunctival fever,[12] even in military recruits,[13] and type 8 represents at least one of the causes of epidemic keratoconjunctivitis.[14,15] Evidence from several reports indicates that serotype 3, and perhaps also 7, may, on occasion, produce corneal lesions not altogether dissimilar to those described in epidemic keratoconjunctivitis.[16]

In their papers given at this symposium, Dr. Ormsby and Dr. Chang mention type 7 in relation to ocular illness. Their agents, when compared in our laboratory to the prototype 7 virus and to a more recently isolated variant which we have tentatively designated 7-prime,[26] show closer relationships to the latter.

Table 1 illustrates the immunologic relationships and differences between type 7 (Gomen) and type 7-prime (S-1058). The Gomen strain was isolated in California by Berge[17] from a military recruit with pharyngitis and fever; S-1058 by Shelokov from a Washington, D.C., child. In addition to the strains of 7-prime from the United States (Shelokov), Canada (Ormsby), and from Saudi-Arabia (Chang), we have received strains from France and England. The latter came from Dr. Pereira and Dr. Tyrrell who recovered them from children ill with pharyngoconjunctival fever in Salisbury and Sheffield, respectively. None of our corrrespondents comments on the occurrence of keratitis.

In this connection, it is interesting to note that Hilleman et al., using paired human

TABLE 1

REPRESENTATIVE CROSS NEUTRALIZATION TEST BETWEEN GOMEN (PROTOTYPE 7) AND S-1058 (TYPE 7-PRIME)

Antiserum (rabbit)	Virus	
	Gomen	S-1058
Gomen	160	5
S-1058	320	1280

serums, reported evidence of immunologic heterogeneity among strains related to type 7.[18]

The principal differences, therefore, between the effects of type 3 and 7 on the one hand, and type 8 on the other, would be the tendency for types 3 and 7 to produce systemic illnesses, with corneal lesions occurring as occasional complications, and the tendency on the part of type 8 not to produce much in the way of systemic illness, but regularly to involve the cornea. The age distribution of the natural occurrence of these different serotypes as well as of the two clinically different illnesses, is also worthy of further study.

There is evidence suggesting that other serotypes are also important in ocular illness; however, it is considerably less than that which exists for types 3, 7, and 8. Part of this evidence derives from observations of naturally occurring illness and part from volunteer studies.

Table 2 briefly tabulates present information available to us on the occurrence of adenovirus types 1, 4, 5, 6, and 10 in cases of naturally occurring illness characterized by conjunctivitis. Obviously, more information must be acquired concerning these types before they can be regarded as definite etiologic agents of ocular illness.

TABLE 2

ADENOVIRUSES TYPED AT NATIONAL INSTITUTES OF HEALTH

(Isolated from cases with conjunctivitis [other than Types 3, 7, and 8].)

Type

1—Three isolations from family outbreaks of fever, pharyngitis, and conjunctivitis—Washington, D. C.[19] One isolation by Dr. Fukumi from child with pharyngoconjunctival fever in Japan.[20]

4—One isolation from a case of follicular conjunctivitis in laboratory worker (RJH).

5—One isolation by Dr. Fukumi from a three year-old child with pharyngoconjunctival fever

6—Two isolations reported by Jawetz.[21] The Washington, D. C., case (a physician) had follicular conjunctivitis; 3 other members of family had pharyngoconjunctival fever.

10—Prototype (Jones) from a case of simple follicular conjunctivitis. Also isolated from a child with conjunctivitis during attack of measles.

VOLUNTEER STUDIES

Rather extensive studies on the effects of adenoviruses in volunteers have been carried out by our group in collaboration with Dr. Thomas Ward of The Johns Hopkins School of Hygiene and Public Health, and most of these experiences have been reported in detail.[22,23] Therefore, we will touch only on certain highlights having to do with ocular disease.

From our early studies, it appeared virtually impossible to produce objectively measurable clinical illness in volunteers by inoculating the adenoviruses into the nose or the throat. On the other hand, the same virus materials which failed to produce disease when inoculated into nose and throat, produced definite, clear-cut, objective illnesses when swabbed on the conjunctiva. Thus, when type 3 and, surprisingly enough, types 1, 4, 5, and 7 adenovirus were swabbed on the conjunctiva, after an incubation of 48 to 72 hours, the succeeding events closely paralleled those of naturally occurring pharyngoconjunctival fever.

The conjunctival mode of inoculation made it possible to reproduce regularly a characteristic, easily recognized clinical syndrome in more than 90 percent of susceptible volunteers. An experimental monovalent type 3 vaccine, inactivated by formalin, was then tested in volunteers. Seventy percent of the volunteers, when challenged three weeks later, appeared protected to an extent equivalent to that provided other volunteers by naturally acquired antibodies.[24]

POSSIBLE IMPORTANCE OF IRRITATION IN THE CLINICAL MANIFESTATIONS OF ADENOVIRUS INFECTION

It has been known for a number of years that keratoconjunctivitis very often followed exposure to some factor capable of causing irritation of the cornea. Thus epidemic keratoconjunctivitis was particularly prevalent during World War II in arc welders employed in the ship-building industry, and, subsequently, in other industrial workers ex-

periencing high rates of ocular foreign bodies. Some of the more recent outbreaks have been traced to tonometers used in ophthalmologic work.[22]

The literature on pharyngoconjunctival fever, including Derrick's observation of "swimming-bath" conjunctivitis in Brisbane, Cockburn's Greeley outbreak, and our own outbreak in northern Virginia, as well as subsequent outbreaks, all indicate that swimming is an important accessory factor either in dissemination of virus or in the genesis of clinical manifestations, or both.

Reasoning by analogy from our volunteer study experiences, we are inclined to think that the irritation of the conjunctiva produced by chlorine or more likely by water alone, followed by rubbing, would be sufficient to account for the apparent potentiating effect. An experience by one of the authors may be worth relating.

He became infected during the autumns of two successive years, 1953 and 1954, with types 3 and 4 adenoviruses, respectively. The illnesses occurred under almost precisely the same circumstances. The onset, in each instance, occurred several days after the corn-picking season had opened, and were first noticed as a "foreign body sensation in the right eye" while picking corn on his farm. The sensation in itself was not unexpected, since he and several of his children have a mild hypersensitivity to corn pollen. However, it turned out, in each instance, that this rather more severe foreign body sensation represented the onset of acute follicular conjunctivitis which lasted more than a week, in both instances due to adenovirus infection. The infections were characterized by a unilateral sore throat with virtually no fever. The conjunctivitis eventually spread to the other eye, but was less severe.

The factor of irritation was subsequently specifically tested directly in volunteers.[23] Of nine susceptible volunteers swabbed on the conjunctiva with type 3 virus, each developed typical pharyngoconjunctival fever. The same virus, given in somewhat larger quantities, but merely dropped on the conjunctiva of 10 other susceptible volunteers, resulted in only two definite and one questionable cases of pharyngoconjunctival fever. This was a double-blind experiment in which one eye in every person was swabbed with either virus or control material, and the other eye received drops of one or the other, and the throat was also swabbed with control material. Furthermore, when materials such as tissue culture fluids were dropped in the eyes of volunteers, it was somewhat difficult to keep the volunteer from subsequently rubbing his eyes, and it is possible that the persons who did develop infection with virus which was dropped in the eye may have, in a sense, provided their own swabbing effect.

Latency or chronicity of adenovirus infection

Any discussion of adenoviruses in the production of human disease would be incomplete without calling attention to the tendency of certain of these agents to persist in the involved tissues. As high as 90 percent of adenoid and tonsil tissues were shown by our group and others to harbor adenoviruses of one type or another, most often type 1, and next most often type 2. Acute infections with these serotypes are most prevalent in childhood, yet, when looked for in adenoids of older persons, they are found as frequently as in children.

It will be of interest to determine in future studies whether or not adenoviruses, like herpes virus and certain other latent virus infections, can cause recrudescent illnesses. Their possible role in recurrent nasopharyngitis, more commonly called "colds," in chronic follicular pharyngitis, tonsillitis, and conjunctivitis, and in recurrent punctate keratitis should be seriously considered. Unfortunately, in limited experiments to date, rises in antibody levels were not demonstrated, and isolations could not be made with regularity from such illnesses. However, by analogy with recurrent herpes labialis, specific antibody responses would not be expected to occur, and antibodies in the

nasal secretions might prevent recovery of the virus.

Summary

Certain adenoviruses, namely types 3, 7-prime, and 8, are well established as causes of ocular disease; types 3 and 7-prime cause chiefly acute follicular conjunctivitis with systemic symptoms and infrequently with corneal opacities, whereas type 8 causes epidemic keratoconjunctivitis, with frequent opacities and rarely with systemic symptoms. Other serotypes have been recovered from occasional cases of conjunctivitis and have produced conjunctivitis in volunteers. Conjunctival irritation appears to play a role in establishment of infection.

National Institutes of Health (14).

References

1. Huebner, R. J., Rowe, W. P., Ward, T. G., Parrott, R. H., and Bell, J. A.: Adenoidal-pharyngeal-conjunctival agents: A newly recognized group of common viruses of the respiratory tract. New England J. Med., **251**:1077-1086, 1954.
2. Rowe, W. P., Huebner, R. J., Hartley, J. W., Ward, T. G., and Parrott, R. H.: Studies of the adenoidal-pharyngeal-conjunctival (APC) group of viruses. Am. J. Hyg., **61**:197-218 (Mar.) 1955.
3. Rowe, W. P., and Huebner, R. J.: Present knowledge of the clinical significance of the adenoidal-pharyngeal-conjunctival group of viruses. Am. J. Trop. Med. & Hyg., **5**:453-460, 1956.
4. Enders, J. F., Bell, J. A., Dingle, J. H., Francis, T., Hilleman, M. R., Huebner, R. J., and Payne, A. M.: Adenoviruses: Group name proposed for new respiratory-tract viruses. Science, **124**:119-120, 1956.
5. Parrott, R. H., Rowe, W. P., Huebner, R. J., Bernton, H. W., and McCullough, N. M.: New England J. Med., **251**:1087-1090, 1954.
6. Bell, J. A., Rowe, W. P. Engler, J. I., Parrott, R. H., and Huebner, R. J.: Pharyngoconjunctival fever: Epidemiological studies of a recently recognized disease entity. J.A.M.A., **157**:1083-1092, 1955.
7. Beal, R.: Ann. d'ocul., Jan., 1907.
8. Tanaka, C.: Epidemic keratoconjunctivitis in Japan and the Orient. (Paper given at Keratoconjunctivitis Symposium, San Francisco, Sept. 7-8, 1956.)
9. Derrick, E. H.: Swimming bath conjunctivitis, with a report of 3 probable cases and a note on its epidemiology. M. J. Australia, **2**:334-336, 1943.
10. Cockburn, T. A.: Epidemic of conjunctivitis in Colorado associated with pharyngitis, muscle pain, and pyrexia. Am. J. Ophth., **36**:1534-1539, 1953.
11. Cockburn, T. A., Rowe, W. P., and Huebner, R. J.: Relationship of the 1951 Greeley, Colorado, outbreak of conjunctivitis and pharyngitis to type 3 APC virus infection. Am. J. Hyg., **63**:250-253, 1956.
12. Pereira, H. G., and Tyrrell, A. J.: Personal communications, 1956.
13. Descomb, H. E., and Hilleman, H. R.: Clinical and laboratory studies in patients with respiratory disease caused by adenoviruses (RI-APC-ARD agents). Am. J. Med., **21**:161-174, 1956.
14. Jawetz, E., Kimura, S., Nicholas, A. N., Thygeson, P. and Hanna, L.: New type of APC virus from epidemic keratoconjunctivitis. Science, **122**:1190-1191, 1955.
15. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., and Kimura, S.: Antibodies to APC virus type 8 in epidemic keratoconjunctivitis. Proc. Soc. Exper. Biol. & Med., **92**:91-95, 1956.
16. Ormsby, H. L., Fowle, A. M. C., and Cockeram, A.: Relation of epidemic keratoconjunctivitis to the adenoidal-pharyngeal-conjunctival (APC) virus syndrome. Canad. M. A. J., **73**:710-713, 1955.
17. Berge, T. O., England, B., Mauris, C., Shuey, H. E., and Lennette, E. H.: Etiology of acute respiratory disease among service personnel at Fort Ord, Calif. Am. J. Hyg., **62**:283-294, 1955.
18. Hilleman, M. R., Werner, J. H., and Stewart, M. T.: Grouping and occurrence of RI (prototype RI-67) viruses. Proc. Soc. Exper. Biol. & Med., **90**:555-562, 1955.
19. Merchant, R. H., and Kasel, J. A.: Personal communication.
20. Fukumi, H.: Personal communication.
21. Jawetz, E., Hanna, L., Kimura, S. J., and Thygeson, P.: A new type of APC virus from follicular conjunctivitis. Am. J. Ophth., **41**:231-234, 1956.
22. Ward, T. G., Huebner, R. J., Rowe, W. P., Ryan, R. W., and Bell, J. A.: Production of pharyngo-conjunctival fever in human volunteers inoculated with APC viruses. Science, **122**:1086-1087, 1955.
23. Bell, J. A., Ward, T. G., Huebner, R. J., Rowe, W. P., Suskind, R. G., and Paffenbarger, R. S., Jr.: Studies of adenoviruses (APC) in volunteers. Am. J. Pub. Health, **46**:1130-1146 (Sept.) 1956.
24. Huebner, R. J., Bell, J. A., Rowe, W. P., Ward, T. G., Suskind, R. G., Hartley, J. W., and Paffenbarger, R. S., Jr.: Studies of adenoidal-pharyngeal-conjunctival vaccines in volunteers. J.A.M.A., **159**: 986-989, 1955.
25. Cockburn, T. A., Nitowsky, H., Robison T., and Cheever, F. S.: Epidemic keratoconjunctivitis: A study of a small epidemic. Am. J. Ophth., **36**:1367-1372, 1953.
26. Rowe, W. P., Hartley, J. W., and Huebner, R. J.: Unpublished data.

STUDIES ON ADENOVIRUS INFECTIONS OF THE EYE IN TORONTO*

A. J. Beale, M.D.,† Frances Doane, B.Sc., and H. L. Ormsby, M.D.
Toronto, Ontario

The APC-RI or adenovirus group were first isolated by Rowe et al.[15] from surgically removed adenoids. Hilleman and Werner[7] independently isolated another member of the group from cases of respiratory disease in military recruits. Since that time many new members of the group have been isolated, and evidence of widespread infection of human beings in the United States, Canada, England, Scandinavia, and Europe has been obtained. Although adenoviruses share a small particle size complement fixing antigen, they can be separated on the basis of virus neutralization tests into at least 14 distinct serologic types.[16]

The exact role of these viruses in human disease remains to be determined, but an impressive amount of work has been done in the short period since their discovery. There is now strong evidence that Types 4 and 7 acute respiratory disease (ARD) in military recruits.[6] Types 3 and 4 have been implicated in pharyngoconjunctival fever, a disease characterized by fever, sore throat, and conjunctivitis.[2] It is of particular interest that transmission of this disease to human volunteers has been achieved with types 3 and 4 virus when the conjunctival sac was inoculated, but not following nasal instillation.[17] It is possible that the conjunctiva may be a portal of entry for other members of the group as suggested by Jawetz.[8] Types 1, 2, 3, 4, 5, 6, 7, 8, and 10 have all been isolated from the conjunctival secretions,[14] but more work is required to establish their etiologic role.

* From The Department of Ophthalmology, University of Toronto, and The Research Institute of The Hospital for Sick Children. Assisted with funds allocated by the Province of Ontario under the National Health Grant Programme of the Department of Health and Welfare, Ottawa, Canada.

† Present address: c/o Public Health Laboratory Service, 38 Old Queen Street, Westminster, London, S.W. 1, England.

At the Hospital for Sick Children, Toronto, we have been able to study three groups of cases during the past year:

1. An epidemic of pharyngoconjunctival fever already described by Ormsby and Aitchison.[10]

2. Cases of conjunctivitis referred to one of us (H. L. O.) from Toronto eye clinics.

3. Cases of conjunctivitis in children admitted to The Hospital for Sick Children.

Experience gained in the investigation of these cases forms the basis of the present report.

Methods

Specimens of eye secretion for virus isolation were stored at −20°C. until inoculated into tissue cultures. Penicillin, 500 mls., and streptomycin, 250 μg./ml., were added.

HeLa cells were grown in 20 percent human serum in yeast-extract medium.[12] Before use they were washed three times with Hanks' balanced salt solution, and maintenance fluid, consisting of 10-percent rabbit serum inactivated at 56°C. for half an hour in Medium 199[9] was added.

The method of preparation of human amnion cells is presented in detail because little has been written about the method since the original description of Zitcer et al.[18] The method is based, in part, on the experience of workers in Boston (Enders, personal communication).

Placentas and membranes are collected into a two liter sterile beaker containing about 300 ml. of phosphate buffered saline.[4] Material from Caesarian sections or normal deliveries can be used, but care must be taken to avoid contamination with antiseptics, so the placenta is taken directly from the patient into the beaker. In the laboratory the amnion is dissected free with forceps in a "sterile" cabinet. This is facilitated by suspending the placenta from the cord.

The pieces of membrane are collected in a

large petri dish containing Hanks' balanced salt solution, and are then spread out with forceps. After washing, the amnion is cut into pieces about two inches square and accumulated in a beaker containing 0.25 percent trypsin in Hanks' balanced salt solution adjusted to pH 7.8. The trypsin and pieces of amnion are then transferred to the continuous trypsinizing apparatus described by Rappaport.[11] Mixing is achieved with a magnetic stirrer.

After a preliminary period of 45 minutes, trypsin is allowed to drop in from a reservoir at 37°C.; cells run out into a flask kept in an iced water bath. Trypsin is allowed to run in dropwise so that a liter is used in about three to four hours. When all the trypsin has run through, the cell suspension is filtered through gauze into 250 ml. centrifuge cups and is centrifuged at 800 rpm for 20 minutes.

The cells are resuspended into Hanks' balanced salt solution using a syringe and 18-gauge needle to disperse the cells. They are then centrifuged at 800 rpm for five minutes in a graduated centrifuge tube. The supernatant fluid is discarded, and the cell suspension is diluted in growth medium consisting of Hanks' balanced salt solution and 0.5-percent lactalbumen with the addition of 20-percent human, horse, or ox serum. The cells are enumerated in a hemocytometer after staining with 0.1-percent crystal violet in 0.1M citric acid. Cells are diluted in growth medium to contain 3 by 10^5 cells per ml., or approximately 1:250 of the packed cell volume.

A quantity of 1.0 ml. is dispensed into tubes which are incubated in a stationary rack at 37°C. in a nearly horizontal position. Sheets of cells grow out in about seven days. Before use the cells are washed by the removal of the nutrient fluid and the addition of 1.5 ml. of Hanks' balanced salt solution.

This procedure is repeated three times and finally cells are maintained in Earle's balanced salt solution with 0.5-percent lactalbumen and 0.1-percent yeast extract and 5.0-percent horse serum. For short-term experiments the horse serum may be omitted. This method has proved generally satisfactory, but occasionally cells have failed to grow for no apparent reason.

Strains were typed using antisera against the prototype adenovirus strains, provided by Dr. R. J. Huebner. The method of preparation of antisera and of typing strains was that described by Rowe et al.[13] Fixation of coverslip cultures and staining with hematoxylin and eosin was by the technique described by Doane et al.[3]

Recognition of virus

The changes produced by adenoviruses in HeLa or human amnion cells may be recognized with a fair degree of confidence in wet preparations under the lower powers of the microscope. Cytopathogenic changes, generally seen first at the edge of the culture, consist in the cells becoming distinctly outlined and containing a "core" of granulation. In the final stages the cells are rounded and clumped together, and eventually fall off the glass surface. During growth in HeLa cells the pH of the fluid tends to become quite acid. These changes can be distinguished, with experience, from those caused by poliomyelitis, Coxsackie B, vaccinia, herpes simplex, and some "orphan" viruses.

The changes may be examined in more detail by the use of cells grown on coverslips, which can be removed, fixed, and stained. This method has much to commend it, and in our hands has afforded a rapid means of identifying herpes simplex virus, for example.[1,3]

Stained preparations of HeLa cells which have been inoculated with large amounts of adenovirus type 3 virus show nuclear inclusions which appear to have a similar consistency to the nucleus, but are more eosinophilic. Frequently, a pale area is seen between the large inclusion and the densely outlined nuclear membrane. The nucleoli are still present at this stage.

As degeneration proceeds, the nuclei be-

come more pyknotic and the cells clump together. The appearances of normal HeLa cells and cells infected with adenovirus type 3 are shown in Figures 1 to 3. Somewhat similar changes are seen in human amnion cells. The appearances of normal cells are shown in Figure 4 and after infection with adenovirus type 3 in Figure 5. The intranuclear inclusions are well seen.

The definitive method for the recognition of a member of the APC group is the demonstration of specific complement fixation with known positive antisera.[15] It is important for this purpose to employ several human sera known to be positive for adenovirus infection in order to avoid nonspecific reactions.

Isolation of virus

Material from 55 patients has been examined by the inoculation of HeLa cell cultures. It can be seen from Table 1 that a total of 31 isolations were made from conjunctival secretions. In addition one case yielded virus from the throat but not from the conjunctival secretions, making a total of 32 isolations. The highest rate of virus isolation was from hospitalized children, eight out of 11 of whom yielded virus.

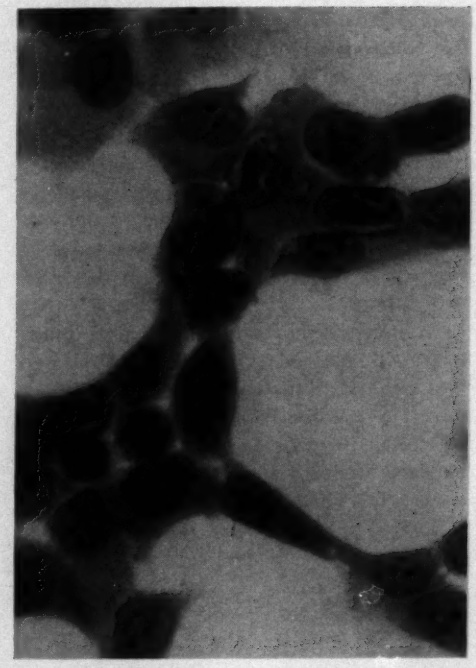

Fig. 2 (Beale, Doane, and Ormsby). HeLa cells 17 hours after infection with adenovirus type 3. Hematoxylin-eosin, ×500.

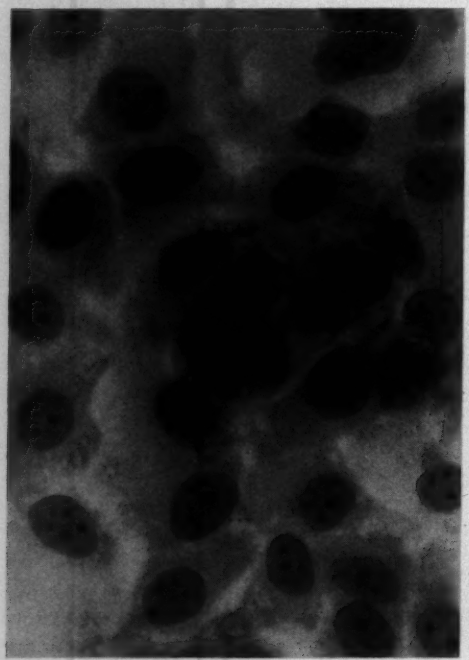

Fig. 1 (Beale, Doane, and Ormsby). Normal HeLa cells. Hematoxylin-eosin, ×500.

These results would appear to show a satisfactory rate of virus isolation from cases of possible adenovirus infection. In fact, however, considerable trouble has been experienced in virus isolation tests, for these viruses are often slow in producing degenerative changes. Great care in the control of the pH of the cultures and frequent changes of nutrient fluid are required to maintain HeLa cells in a healthy condition for long periods.

As has been pointed out by Ginsberg,[5] adenoviruses are slowly absorbed to and released from HeLa cells, and this probably

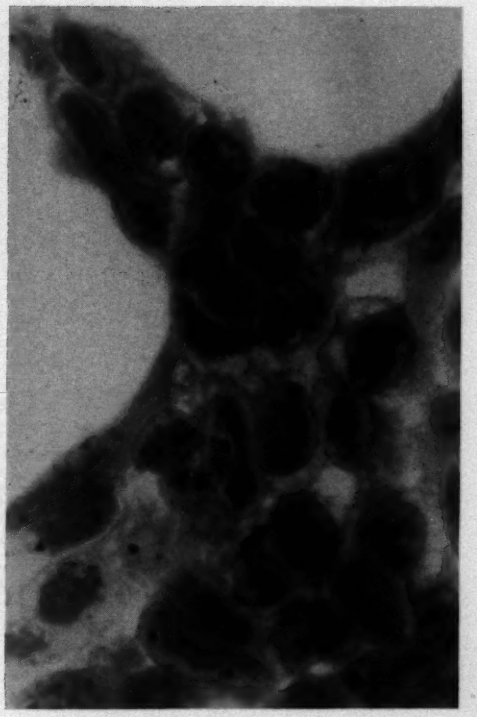

Fig. 3 (Beale, Doane, and Ormsby). HeLa cells 25 hours after infection with adenovirus type 3. Hematoxylin-eosin, ×500.

explains the marked delay in the onset of visible cytopathogenic changes that is noted when small amounts of virus are inoculated.

If a subculture is made when the quality of the HeLa cells is too bad to allow changes to be certainly identified, the number of isolations is greatly increased. Seven of the 32 isolations were achieved by such a subculture. The rapidity with which virus action became apparent on the second passage indicated that virus had multiplied in the cells during the first passage. Intracellular virus was probably released during the freezing and thawing resulting from storage prior to subculture.

Recently we have used human amnion tissue in our laboratory, and it appears to be superior to HeLa cells. Thus, in comparative tests using HeLa and human amnion cells for primary isolation, changes have appeared earlier and have been more easily detectable in human amnion cultures. The time taken for the appearance of cytopathogenic changes in parallel isolations on seven patients is shown in Table 2. It will be seen that with five patients changes appeared earlier in amnion cells. One patient, 29, failed to yield a virus in HeLa cells, but since the virus had been isolated in these cells six months earlier, it would probably have been evident on subculture. The most striking improvement is in the quality of cells over long periods of maintenance, rather than any great increase in sensitivity. This allows minimal cytopathogenic changes to be recognized with confidence, even when cultures have been maintained without fluid change for as long as one month.

Typing of strains

At least 14 distinct types of adenovirus

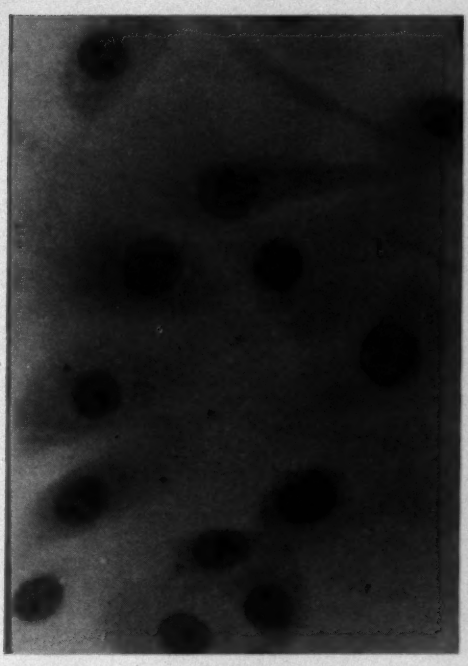

Fig. 4 (Beale, Doane, and Ormsby). Normal human amnion cells. Hematoxylin-eosin, ×500.

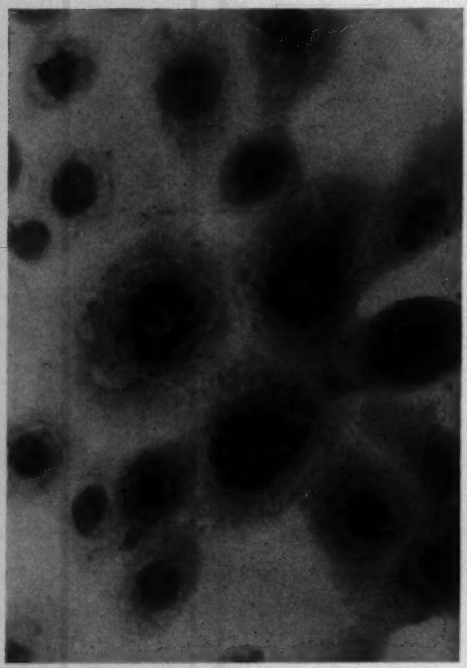

Fig. 5 (Beale, Doane, and Ormsby). Human amnion cells infected with adenovirus type 3. Hematoxylin-eosin, ×500.

are now recognized, and it is likely that additional types will be identified. The task of keeping on hand antisera to all the serotypes is beyond the capacity of all but specialist laboratories. Fortunately, for most purposes the complement fixation test with the group antigen is adequate. Typing is required, however, in epidemiologic studies, and when attempting to associate particular clinical entities with particular serotypes. Present evidence indicates that several serotypes may be associated with the same clinical entity. Typing is likely to be of greatest value in tracing the spread of epidemics, and such work might be profitably concentrated in a few centralized laboratories.

The results of typing the 32 agents isolated in Toronto are presented in Table 3. It will be seen that all the strains isolated from the epidemic of pharyngoconjunctival fever described by Ormsby and Aitchison[10] were type 7. This presumably means that this type, in addition to types 3 and 4, may cause this syndrome. Fifteen type 3 and 11 type 7 strains were isolated from the adults and children with conjunctivitis. In addition one patient yielded type 2 virus and one type 9

TABLE 1

ADENOVIRUS INFECTIONS OF THE EYE, TORONTO 1955
VIRUS ISOLATIONS IN HeLa CELLS

Category of Case	Number Tested	Virus Isolated
Epidemic of pharyngoconjunctival fever	8	4*
Toronto Eye Clinics	36	20
Hospital for Sick Children cases	11	8
Total	55	32

* Includes one case yielding virus only from the throat.

TABLE 2

ADENOVIRUS INFECTIONS OF THE EYE, TORONTO, 1955
ISOLATION OF VIRUS IN HeLa AND AMNION CELLS

Case Number	Days Required to Produce Infection	
	HeLa	Amnion
9	8	7
27	7	7
29	—*	7
46	8	7
50	7	7
58	11	8
67	11	8

* Virus isolated in HeLa cells six months earlier.

TABLE 3

ADENOVIRUS INFECTIONS OF THE EYE, TORONTO 1955
ANTIGENIC TYPES OF VIRUS ISOLATED

Category of Case	Number Tested	Type
Epidemic of pharyngojunctival fever	4	4 Type 7
Toronto Eye Clinics	20	1 Type 2; 12 Type 3; 6 Type 7; 1 Type 9.
Hospital for Sick Children cases	8	3 Type 3; 5 Type 7

virus. Some patients infected with either type 3 or type 7 virus showed corneal opacities when examined with a slitlamp.

Many of the type 7 strains have shown partial neutralization with type 3 antiserum also. This suggests that the two types may be more closely related to each other than to the other members of the group.

Summary and Conclusions

The adenoviruses are an important new group of agents. Our results show that human amnion cells may be superior to HeLa cells for the study of these viruses.

Adenoviruses have been implicated in at least three forms of conjunctivitis: (1) Pharyngoconjunctival fever; (2) acute follicular conjunctivitis; (3) epidemic keratoconjunctivitis. Their exact role in these diseases and the relationship of particular serotypes to specific clinical states require more study. Type 7 virus has been isolated from patients in an epidemic of pharyngoconjunctival fever associated with swimming pools in Toronto. Types 2, 3, 7, and 9 virus were isolated from cases of conjunctivitis in adults and children.

Banting Institute (5).

Our thanks are due to numerous Toronto physicians who have referred cases to us. Special thanks are due to Dr. R. J. Huebner, who not only provided us with the protype strains of adenovirus, but also allowed one of us (F. D.) to observe the methods used in his laboratory. We are also indebted to Dr. A. J. Rhodes for his constant advice and encouragement.

References

1. Beale, A. J., and Hair, H. C.: Rapid diagnosis of the herpetic variety of Kaposi's varicelliform eruption by tissue culture methods. Canad. M. A. J., **74**:443-448, 1956.

2. Bell, J. A., Rowe, W. P., Engler, J. I., Parrott, R. H., and Huebner, R. J.: Pharyngoconjunctival fever: Epidemiological studies of a recently recognized disease entity. J.A.M.A., **157**:1083-1092, 1955.

3. Doane, F., Rhodes, A. J., and Ormsby, H. L.: Rapid diagnosis of herpetic infections by isolation of virus in tissue cultures. Can. M. A. J., **73**:260-263, 1955.

4. Dulbecco, R., and Vogt, M.: Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exper. Med., **99**:167-182, 1954.

5. Ginsberg, H. S.: 1956 conference on viruses in search of disease. Ann. New York Acad. Sci., in press.

6. Hilleman, M. R.: 1956 conference on viruses in search of disease. Ann. New York Acad. Sci., in press.

7. Hilleman, M. R., and Werner, J. H.: Recovery of new agent from patients with acute respiratory illness. Proc. Soc. Exper. Biol. & Med., **85**:183-188, 1954.

8. Jawetz, E.: 1956 conference on viruses in search of disease. Ann. New York Acad. Sc., in press.

9. Morgan, J. F., Morton, H. J., and Parker, R. C.: Nutrition of animal cells in tissue cultures: 1. Initial studies on a synthetic medium. Proc. Soc. Exper. Biol. & Med., **73**:1-8, 1950.

10. Ormsby, H. L., and Aitchison, W. S.: The role of the swimming pool in the transmission of pharyngeal-conjunctival fever. Canad. M. A. J., **73**:864-866, 1955.

11. Rappaport, C.: Trypsinization of monkey-kidney tissue: An automatic method for the preparation of cell suspensions. Bull. World Health Organ., **14**:147-166, 1956.

12. Robertson, H. E., Brunner, K. T., and Syverton, J. T.: Propagation in vitro of poliomyelitis viruses: VII. pH change of HeLa cell cultures for assay. Proc. Soc. Exper. Biol. & Med., **88**:119-122, 1955.

13. Rowe, W. P., Huebner, R. J., Hartley, J. W., Ward, T. G., and Parrott, R. H.: Studies of the adenoidal-pharyngeal-conjunctival (APC) group of viruses. Am. J. Hyg., **61**:197-218, 1955.

14. Rowe, W. P.: 1956 conference on viruses in search of disease. Ann. New York Acad. Sc., in press.

15. Rowe, W. P., Huebner, R. J., Gilmore, L. K., Parrott, R. H., and Ward, T. G.: Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exper. Biol. & Med., **84**:570-573, 1953.

16. Rowe, W. P., Hartley, J. W., and Huebner, R. J.: Additional serotypes of the APC virus group. Proc. Soc. Exper. Biol. & Med., **91**:260-262, 1956.

17. Ward, T. G., Huebner, R. J., Rowe, W. P., Ryan, R. W., and Bell, J. A.: Production of pharyngoconjunctival fever in human volunteers inoculated with APC viruses. Science, **122**:1086-1087, 1955.

18. Zitcer, E. M., Fogh, J., and Dunnebacke, T. H.: Human amnion cells for large-scale production of polio virus. Science, **122**:30, 1955.

AGENTS RECOVERED FROM ACUTE CONJUNCTIVITIS CASES IN SAUDI-ARABIA*

E. S. Murray, M.D., R. S. Chang, M.D., S. D. Bell, M.D.,
M. L. Tarizzo, M.D., and J. C. Snyder, M.D.
Boston, Massachusetts

Abstract

Epidemiologic studies of trachoma in villages of eastern Saudi-Arabia were initiated during the year. Approximately 1,500 people in 19 different places have been examined. Specimens were obtained from the eyes of approximately 1,000 persons for microscopic study. Most of those examined were under 15 years of age. Findings consistent with trachoma were obtained in more than 90 percent of the subjects of school age or older. Inclusions were observed in about 15 percent.

Intensive efforts were made to isolate agents from conjunctival scrapings of patients believed to have early trachoma. Human conjunctival cells in tissue culture were used for most of the trials, but other cell lines were tested in a few instances. The experiments were greatly complicated by the unsuspected presence of a minute coccobacillary contaminant in certain components of the culture media.

Between July, 1955, and March, 1956, approximately 600 conjunctival scrapings were obtained from Saudi-Arabia. Of these, nearly 200 have now been tested, yielding 13 viruses, at present tentatively classified as follows: One Coxsackie virus, Group B-1; 12 viruses of the adenovirus group (APC). Of the 12 adenoviruses, one strain has not been screened but probably is one of the very common, widely distributed viruses. The remaining 11 have been screened with the cooperation of Dr. Huebner: one each of types 3, 7, and 8 are now recognized, and seven of the eight viruses which do not correspond to any known type have been shown to fall into four immunologic groups. The etiologic relationship of the untyped agents to clinical trachoma is not clear as yet. Serologic evidence and probably also tests in man will be necessary to evaluate these viruses; inclusions have not been found in cells attacked by these agents in vitro.

Scrapings from trachomatous eyes were inoculated into a baby chimpanzee on two different occasions but no clinical manifestations developed.

One Shattuck Street (15).

* From the Harvard University School of Public Health.

ADENOVIRUSES FROM CANADIAN CASES OF KERATOCONJUNCTIVITIS*

Ann M. Fowle, Ph.D., Violet Simmons, and H. L. Ormsby, M.D.
Toronto, Ontario

The isolation of four strains of adenovirus type 3 from patients with keratoconjunctivitis has already been reported from this laboratory (Fowle, Cockeram, and Ormsby, 1955[2]). Although these strains belonged to a single type antigenically, the corneal involvement varied from transient nebulous epithelial opacities to fairly marked disclike opacities similar to those seen in epidemic keratoconjunctivitis (EKC). In this study, a further comparison was made of these strains of virus by means of cross neutralization tests using hyperimmune rabbit sera. The results, however, showed that there was not an antigenic difference be-

* From the Departments of Ophthalmology and Bacteriology, Faculty of Medicine, University of Toronto.

tween any two of the strains of 50 percent or greater, a difference which has been considered to be significant (Archetti and Horsfall, 1951[1]). The data rather suggest a very close similarity despite the clinical variation.

Materials and Methods

Corneal involvement

Although the case histories of the patients have been described elsewhere (Fowle, Cockeram, and Ormsby, 1955) they will be briefly summarized here since a description of each patient reveals the variation in corneal manifestations:

1. Patient I. A., aged 23 years, developed a sore right eye nine days after swimming in a swimming pool. There was moderate follicular formation, preauricular adenopathy, and some velvety pseudomembranes over the palpebral conjunctiva in both upper and lower lids. On the fifth day after onset, two small punctate staining areas were seen in the epithelium at the pupillary margin. There was diffuse stromal thickening deep to Bowman's membrane in these areas and the patient was conscious of a haziness of his vision. These opacities were nebulous and did not resemble those of epidemic keratoconjunctivitis. The second eye became involved in a similar process, five days after onset in the first, but no corneal changes developed. The opacities rapidly faded and had completely disappeared within three weeks of their appearance.

2. Patient H. O., a man, aged 43 years, was inoculated with tissue culture fluid from the fourth passage in monkey-kidney epithelium of virus material from Patient I. A. Five days and 12 hours after this inoculation a foreign body reaction was experienced in the inoculated eye and a follicular conjunctivitis with marked tearing and fine velvety pseudomembranes developed. Five days and 12 hours after the onset in the first eye a similar but less severe conjunctivitis developed in the left eye. Three nebulous diffuse subepithelial opacities were seen on slitlamp examination by a number of ophthalmologists in the first eye and two in the second eye. These were near the visual axis and caused some blurring of vision. Two months later one opacity was still evident in each eye, but could only be seen by slitlamp examination. Subsequent examination on the third month revealed no evidence of any opacities.

3. Patient W. W., a male intern, aged 23 years, developed a severe follicular conjunctivitis in the right eye associated with moderate preauricular adenopathy. Because of his proximity in the hospital, it was possible to study the various stages of the disease at regular intervals. Three days after the onset in the right eye, the left eye became involved and the severity of the process was only slightly less acute in the second eye. During the second week, a number of epithelial erosions developed in both eyes, and on the following week a number of fairly sharply circumscribed subepithelial opacities were seen. These opacities were similar to those seen in epidemic keratoconjunctivitis in their distribution about the visual axis and in the deep layers of the epithelium and beneath Bowman's membrane. Following the subsidence of the acute phase of the disease these opacities began to fade and no longer obscured vision. However, on slitlamp examination six months after onset, two opacities could still be seen.

4. Patient A. B., a man, aged 64 years, was first examined three days after the onset of a severe follicular conjunctivitis in the left eye which was associated with marked regional adenopathy. A milder but similar infection developed in the right eye five days after the onset in the left. On the seventh day after the onset, a number of punctate epithelial staining areas were seen in the left cornea. On the 18th day after the onset he was re-examined and there were approximately 15 subepithelial opacities in each eye, many of which were directly beneath punctate lesions in the epithelium. Some stromal edema was present in both corneas and the visual acuity was reduced to 20/50 in the left eye and 20/40 in the right. The opacities were not sharply circumscribed and began

to fade rapidly as the acute phase of the disease passed. Vision returned to normal within six weeks after the onset and a few opacities could still be seen with the slitlamp three months after onset.

Tissue cultures

The strains of virus were propagated in roller tube cultures of monkey-kidney epithelium obtained from the Connaught Medical Research Laboratories, Toronto. The cells were maintained in 1.0 ml. of synthetic medium No. 597 containing streptomycin and penicillin and buffered to pH 7.2 with 2.8-percent sodium bicarbonate. The four strains of virus which had been isolated in HeLa cell tissue cultures, were passed in monkey-kidney epithelium until the incubation period for a complete cytopathogenic effective became minimum and consistent, at usually four or five days. A relatively low titer of virus was obtained in monkey-kidney epithelium as compared with HeLa cell cultures and therefore the virus was always used undiluted in the neutralization tests. These tests were carried out using cultures of monkey-kidney epithelium but the tubes remained stationary in a 37°C. incubator.

Sera

Specific antisera for neutralization tests were prepared against the viable virus of each strain in young adult albino rabbits. Pooled supernatant fluid from cultures of monkey-kidney epithelium showing a complete CPE, was preserved by freezing at −20°C. Two rabbits were immunized against each strain of virus using the following procedure; 1.5 ml. aliquots of tissue culture fluid were inoculated intravenously twice a week for three weeks. One week after the sixth inoculation the rabbits were test bled and two further inoculations were given. One week after the eighth inoculation, the rabbits were bled again. Usually an increase in titer was obtained using eight inoculations. When two additional inoculations were given, no further increase in titer was obtained. Therefore routinely eight inoculations were given and the rabbits were exsanguinated by cardiac puncture one week after the eighth dose. The sera were stored frozen in 4.0 ml. amounts at −20°C., without preservative. For use in neutralization tests all sera were heated at 56°C. for 30 minutes before being mixed with virus.

Neutralization tests

The tests were carried out as previously described (Fowle, Cockeram, and Ormsby, 1955[2]) using a constant dilution of virus and serial twofold dilutions of serum over the range 1:64 to 1:4,096. Five monkey-kidney roller tubes were inoculated with each dilution. The following controls were done with each experiment—normal control using normal rabbit serum, serum control for each serum used, and virus control for each strain of virus used. The end-point was taken 48 hours after the virus controls showed a complete CPE. First the sera from the paired rabbits were tested against the homologous virus. In only one instance (I. A.) was there a difference in titer and in this case the one with the higher titer was later used against the heterologous strains of virus. In these tests a given strain of virus was tested simultaneously against all four sera. The CPD_{50} was calculated using the Karber method and logarithms to the base 2.

Results

The results of the neutralization tests are summarized in Table 1. In order to test for differences in antigenic composition the formula of Archetti and Horsfall (1951[1]) was applied to the data. Thus the ratios R_1 and R_2 which represent the ratios of the heterologous to homologous titers, were calculated for each of the two antisera and the corresponding antigens. And from these data the significance of the antigenic difference, R, was calculated where $R = R_1 \times R_2$. These ratios are given in Table 2. If $R = 1$, there is no significant difference. If $R = \frac{1}{2}$ or less, there is an antigenic difference of 50 percent or greater between the two antigens. The results of these experiments indicate

TABLE 1

THE NEUTRALIZATION IN TISSUE CULTURE OF STRAINS OF VIRUS BY HOMOLOGOUS AND HETEROLOGOUS RABBIT ANTISERA

Virus Strain	Reciprocal of Serum Dilution Giving 50-percent Protection against Cytopathogenic Effect (CPD_{50})			
	I. A.	H. O.	W. W.	A. B.
I. A.	1003	891	1003	962
H. O.	1458	1499	1105	1217
W. W.	2210	1332	1010	1489
A. B.	1128	648	760	1112

TABLE 2

CALCULATION OF THE SIGNIFICANCE OF ANTIGENIC DIFFERENCE BETWEEN THE STRAINS OF VIRUS

Virus Strain	Values R_1* and R_2*			
	I. A.	H. O.	W. W.	A. B.
I. A.	1	1/1.12	1	1/1.04
H. O.	1/1.03	1	1/1.36	1/1.23
W. W.	2.19	1.32	1	1.47
A. B.	1.01	1/1.72	1/1.46	1

*R_1—divide the heterologous titer obtained with virus 2 by the homologous titer obtained with virus 1.
*R_2—divide the heterologous titer obtained with virus 1 by the homologous titer obtained with virus 2

Virus Strains	$R = \sqrt{R_1 \times R_2}$
I. A. vs. H. O.	1/1.1
I. A. vs. W. W.	1.4
I. A. vs. A. B.	1.0
H. O. vs. W. W.	1.0
H. O. vs. A. B.	1/1.5
W. W. vs. A. B.	1.0

that there is no significant difference in antigenic composition of any two of the strains investigated. In fact, the data suggest a very close similarity, three pairs showing an R value of 1.

SUMMARY AND CONCLUSIONS

1. Four strains of virus isolated from patients with corneal involvement, belonging to adenovirus type 3, were studied in cross neutralization tests.

2. Corneal changes in these four patients varied. In one, transient nebulous stromal opacities appeared after the eighth day of the disease and faded within six weeks. In the second patient, inoculated with virus from the first patient, a similar type of opacity was observed. In the third, the opacities could not be distinguished from the disc-like opacities characteristic of epidemic keratoconjunctivitis, but persisted for only six months. In the fourth patient, a diffuse stromal edema reduced the vision to 20/50 for three months after the acute phase of the disease had subsided.

3. Results of the cross neutralization tests indicated a very close antigenic similarity of all four strains of virus.

Banting Institute (5).

REFERENCES

1. Archetti, I., and Horsfall, F. L.: Persistent antigenic variation of influenza viruses after incomplete neutralization in ovo with heterologous immune serum. J. Exper. Med., **92**:441-462, 1950.

2. Fowle, A. M. C., Cockeram, A., and Ormsby, H. L.: Virus isolations from patients with keratoconjunctivitis. Am. J. Ophth., **40**:180-188 (Nov. Pt. II) 1955.

EPIDEMIOLOGY OF PHARYNGOCONJUNCTIVAL FEVER*

JOSEPH A. BELL, M.D.
Bethesda, Maryland

This discussion of the epidemiology of pharyngoconjunctival fever is generally limited to the adenovirus type 3 disease observed by our research group in the Washington, D.C., area.[1-4] The group includes Dr. R. J. Huebner, Dr. W. P. Rowe, Dr. R. G. Suskind, Dr. R. H. Parrott, Dr. R. W. Ryan, Dr. J. I. Engler, and Dr. J. A. Bell. The virus is readily recoverable in HeLa cell tissue culture from conjunctival lesions and from throat and anal swabs collected from humans ill with the disease. Although the virus has not commonly been found in such specimens from well persons, it has not infrequently been found in an apparent latent state in tonsil and adenoid tissue surgically removed from children. All present evidence is consistent with an hypothesis that humans constitute the chief reservoir of infection.

The isolation of type 3 virus from a laboratory worker with conjunctivitis in early 1954 led to the recognition of an outbreak of eight cases in the Clinical Center Hospital[1] in February and within a few months this led to the observation of some 400 cases occurring sporadically and in localized outbreaks.[2] Chart 1 illustrates the time occurrence of the cases we observed in the summer of 1954.

Group 1 represents cases which occurred in a summer day camp where 20 supervisors entertained 180 four to 12-year old campers, six hours a day, five days a week, for six weeks. Wading or swimming was one of the chief occupations. One hundred and forty-seven cases occurred and 45 percent of these had conjunctivitis.

The children had 435 family household associates and 81 of these were attacked (group 2) but only 22 percent had conjunctivitis.

The Broyhill residential area (group 3) had a neighborhood swimming pool and we observed 25 cases in this area, enough to assure us that an outbreak existed. No direct connection could be found between these cases and the Burgundy Camp outbreak.

The early cases in the Hollin Hall residential area (group 4) were neighborhood playmates of Burgundy Camp cases. This area was placed under epidemiologic surveillance because a new swimming pool was to be opened in late August. It opened a few days before school opened on September 1st, and an outbreak of 104 cases ensued, 44 percent of which had conjunctivitis.

The 25 sporadic cases (group 5) were of widespread distribution and had no direct connection with any of the above cases. During the winter and summer of 1955, many cases came to our attention. In December, 1954, at a Washington, D.C., orphanage, we began medical surveillance of a changing population group of approximately 60 children between the ages of six months and two years. An outbreak occurred with a peak in January, 1956, and type 3 adenovirus was isolated from 38 of these children. Another outbreak occurred with its peak in April, 1956, and type 3 adenovirus was isolated from 28 newly admitted children.

From all these experiences, we must conclude that pharyngoconjunctival fever occurs in all age groups but predominantly in children; it occurs at all seasons of the year; it occurs in epidemic outbreaks particularly in the summer time and commonly associated with swimming; and it occurs either or both endemically and sporadically.

The incubation period is illustrated by Chart 2. It shows the days' interval from onset of first case in a household to onset

* From the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

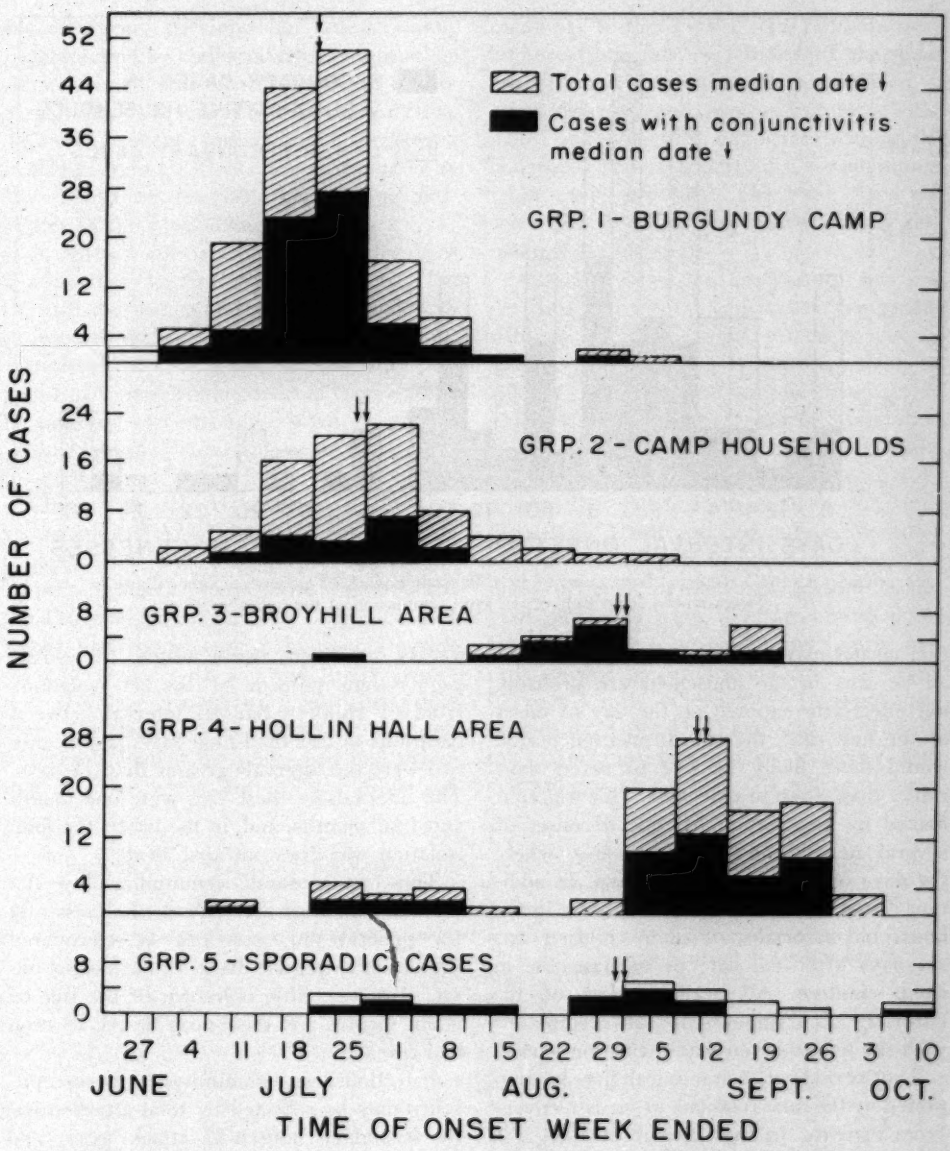

Chart 1 (Bell). Time occurrence of illnesses according to epidemiologic group. (Reproduced from Bell, et al.,[3] with permission of the publisher.)

of other cases. It will be noted that the distribution is similar in virus-positive and other households, many of which were not tested. The first case was generally a camp child and it is reasonable to believe that the cases occurring during the first few days are instances of extrahousehold exposure. The distribution suggests that the incuba-

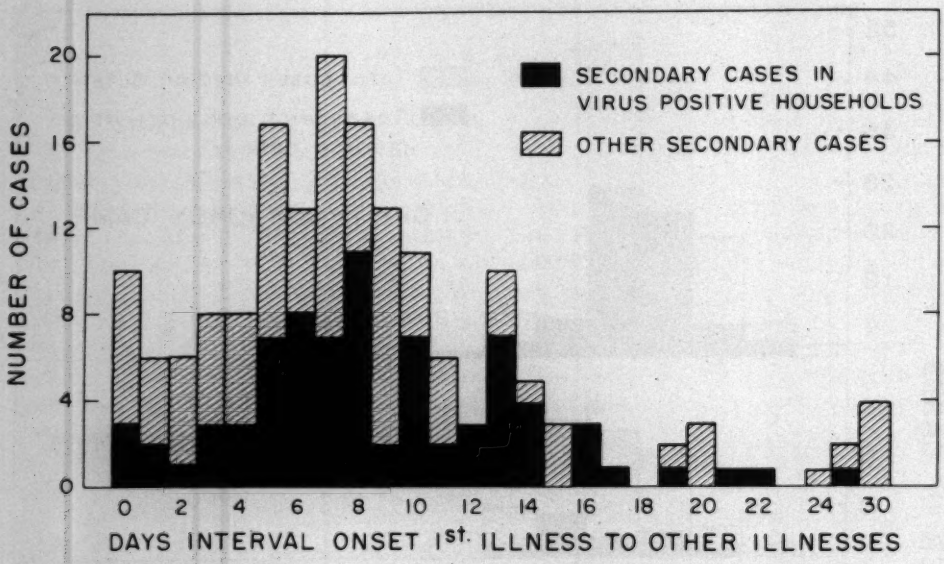

Chart 2 (Bell). Interval from onset of first illness in household to onset of other illnesses. (Reproduced from Bell, et al.,[3] with permission of the publisher.)

tion period may be five to nine days. Since all persons in the household are probably not effectively exposed on the day of onset of the first case, the true incubation period would more likely be five to seven days rather than seven to nine days. This was confirmed by observation of time of onset of several neighborhood contact cases where the days of exposure were known. In addition it was noted that the median case in the household associates of camp children was six days after the date of median case in camp children. All available data on the naturally occurring disease are compatible with the five to seven day incubation period.

The period of communicability is suggested by the time relations of virus recovery from patients. In the summer of 1954, 186 well and ill persons were tested for virus. Virus was isolated from 80 ill persons, and from no well person even though many had intimate contact with typical cases. Seventy-eight of the 80 isolations were during the first nine days of illness.

During the January and April, 1956, outbreaks in a Washington, D.C., orphanage, weekly specimens were tested for virus. Eighty-eight percent of the 98 isolations from 61 children had an interval between isolations of less than nine days, and in only two were the intervals greater than 15 days. The intervals in these two were one month and four months, and, in the latter, the final isolation was from an anal swab.

Thus, in general, communicability decreases with time after onset of illness and for general purposes may be considered slight and negligible 10 to 15 days after onset. However, little is known of the role of latent viruses and their possible reactivation and spread.

Infectiousness, immunity, and susceptibility may be reflected by total attack rates, by secondary household attack rates, and by artificial inoculation of volunteers. By the methods and with the adenoviruses used, we were unable to induce illness in susceptible volunteers through inoculation of live virus by intranasal instillation, by swabbing the oropharynx, or by inhalation of atomized suspension of adenovirus.[5,6]

On the other hand, 90 percent of suscepti-

ble volunteers developed an illness, chiefly characterized by conjunctivitis, two to seven days following the swabbing of live adenovirus types 1, 3, 4, and 5 on the conjunctiva. This 90 percent susceptibility level to a challenge-induced illness was determined by the absence of detectable neutralizing antibodies in the prechallenge serum.

A heat or formaldehyde-inactivated type 3 adenovirus vaccine induced the formation of such antibodies and was associated with protection against the challenge-induced infection and illness.[7] Polyvalent vaccines prepared in a similar manner have been studied in military recruits and shown to protect against naturally occurring, acute febrile respiratory illness.[8,9]

Children in the Washington, D.C., area apparently had a high degree of susceptibility to type 3 infection. This is evidenced by the 70 percent attack rate in those who attended the Burgundy Summer Day Camp. Table 1 shows that the secondary household attack rates decreased with age and this indicates that older age groups were less susceptible than children. This is compatible with an hypothesis that many adults had had infection in prior years and enjoyed prolonged immunity to the disease. The comparatively higher attack rate in females of the older age group is interesting. It is paralleled in other diseases, and may reflect more intimate and effective exposure within the household or less opportunity for immunity from prior exposure. The total cases observed in all areas were nearly equally distributed by sex.

Although extensive analyses of our experience gave results which were compatible with an hypothesis of a person to person spread of the disease, it was impossible to rule out the swimming pool as an accessory mode of spread. The frequent association of summer epidemics with swimming pools needs further study. There is evidence suggesting that purified suspensions of adenovirus type 3 will not long survive in water with 0.4 to 0.6 ppm residual chlorine. The pools involved in the Broyhill and Hollin Hall outbreaks were new swimming pools with automatic chlorination and hourly records showed no residual chlorine fall below 0.6 ppm. On the other hand, a personal communication from Dr. Larson of the Hamilton, Montana, laboratories indicates that cytopathogenic agents resembling adeno-

TABLE 1

SECONDARY HOUSEHOLD ATTACK RATES DURING THREE WEEK PERIOD FOLLOWING ONSET FIRST HOUSEHOLD CASE, BY AGE AND SEX

(Reproduced from Bell, et. al.,[2] with permission of the publisher.)

	Age	Sex	1st Household Case (Excluded)	Persons Exposed to 1st Household Case		
				Persons	Illnesses	Percent Attacked
	0–4		13	63	23	36
	5–9		112	78	50	64
Subtotal	0–9	M	68	80	40	50
		F	57	61	33	54
	10–19		30	52	17	33
	20–19		17	206	31	15
	40+		4	126	8	6
Subtotal	10+	M	29	185	16	9
		F	22	199	40	20
	Total		176	525	129	24

viruses have been recovered from swimming pools. At present it is unknown if contaminated swimming pool water may be a source of epidemic pharyngoconjunctival fever.

Summary

In summary, the disease occurred in all age groups but predominantly in children, and in both sexes, and it occurred in localized epidemic outbreaks and in endemic or sporadic form. The mode of occurrence suggested that (1) infected human beings were a common source of infection, (2) infection generally produces illness, (3) healthy carriers were not an important source of infection in epidemics, (4) the disease was rather highly infectious for young contacts, (5) older persons were more likely than children to be immune presumably from previous attack, (6) contaminated swimming pools are a suspected but unproved source of infection.

The observations indicate that the incubation period is probably five or six days and that the period of communicability as indicated by presence of virus decreases from 100 percent during the first few days of illness to practically nil 10 to 15 days after onset. The disease has not been known long enough to estimate its general prevalence; it has been recognized since 1943 and has occurred in England, Canada, and the United States and perhaps elsewhere. It has occurred in the winter with perhaps a milder type of conjunctivitis, and it is probably a common, widespread respiratory disease. No studies were carried out on treatment, but we gained the clinical impression that common antibiotics did not influence the course of the disease. Infection and disease may be prevented to a substantial extent by vaccine but at present vaccine is not available for general use and should not be considered except in special epidemiologic circumstances.

National Institutes of Health (14).

References

1. Parrott, R. H., Rowe, W. P., Huebner, R. J., Bernton, H.W., and McCullough, N. M.: Outbreak of febrile pharyngitis and conjunctivitis associated with type 3 adenoidal-pharyngeal-conjunctival virus infection. New England J. Med., **251**:1087-1090 (Dec.), 1954.

2. Bell, J. A., Rowe, W. P., Engler, J. I., Parrott, R. H., and Huebner, R. J.: Pharyngoconjunctival fever: Epidemiological studies of a recently recognized disease entity. J.A.M.A., **157**:1083-1092 (Mar.) 1955.

3. Ryan, R. W., O'Rourke, J. F., and Iser, G.: Conjunctivitis in adenoidal-pharyngeal-conjunctival virus infection: Pharyngoconjunctival fever: A newly described entity. Arch. Ophth., **54**:211-216 (Aug.) 1955.

4. Unpublished data.

5. Bell, J. A., Ward, T. G., Huebner, R. J., Rowe, W. P., Suskind, R. G., and Paffenbarger, R. S., Jr.: Studies of adenoviruses (APC)* in volunteers. Am. J. Pub. Health, **46**:1130-1146 (Sept.) 1956.

6. Ward, T. G., Huebner, R. J., Rowe, W. P., Ryan, R. W., and Bell, J. A.: Production of pharyngoconjunctival fever in human volunteers inoculated with APC virus. Science, **122**:1086-1087, 1955.

7. Huebner, R. J., Bell, J. A., Rowe, W. P., Ward, T. G., Suskind, R. G., Hartley, J. W., and Paffenbarger, R. S., Jr.: Studies of adenoidal-pharyngeal-conjunctival vaccines in volunteers. J.A.M.A., **159**: 986-989 (Nov.) 1955.

8. Hilleman, M. R., Stallones, R. A., Gauld, R. L., Warfield, M. S., and Anderson, S. A.: Prevention of acute respiratory illness in recruits by adenovirus (RI-APC-ARD) vaccine. Proc. Soc. Exper. Biol. & Med., **92**:377-383 (June) 1956.

9. Bell, J. A., Hantover, M. J., Huebner, R. J., and Loosli, C. G.: Efficacy of trivalent adenovirus (APC) vaccine in naval recruits. J.A.M.A., **161**:1521-1525 (Aug.) 1956.

KERATOCONJUNCTIVITIS*

The clinical characteristics of the California epidemic, 1941-1942

Michael J. Hogan, M.D.
San Francisco, California

The San Francisco Bay area outbreak of epidemic keratoconjunctivitis developed insidiously in many offices and clinics and most ophthalmologists were treating cases without being aware of the true nature of the disease. Within two to three weeks the increasing number of cases seen each day caused everyone to become alert with regard to this new entity. It lasted from about September, 1941, to February, 1942. Initially most of the patients were shipyard workers but, as the epidemic progressed, more and more nonshipyard workers were seen.

The disease was transmitted along the Pacific Coast in the various shipyards, probably by transfer of personnel, and spread in the various dispensaries. It seemed to start almost simultaneously in the Portland, Los Angeles, and San Francisco Bay area yards. Originally it was thought that welding hoods, goggles, and so forth, might spread the disease, but the appearance in those not utilizing such equipment soon eliminated this theory. The frequency of foreign bodies in welders and chippers and the many visits to dispensaries for treatment seemed to be the principal source of spread. It was soon realized that ocular solutions provided the principal medium for transfer of the infection. It was surprising that probably no more than two or three percent of the workers transmitted the disease to one or more members of their families. Most workers were given ointments to use, and probably treated themselves. Also they were cautioned about the use of towels, wash cloths, and so forth, in their homes. The incidence of transmittal may have been low because of these precautions.

Also, a number of new cases developed among our private and clinic patients as the result of accidental transfer from contaminated solutions, instruments, and hands. Several hundred cases were treated, but an additional number of cases were given medication and advised to remain home until they were past the acute phase. At that time we reported* the results of detailed studies made on 125 of these cases in which the patients were seen and examined throughout the course of the disease.

It is interesting that the epidemic subsided fairly rapidly during February, 1942, and we have speculated at length since that time why it disappeared so completely as an epidemic. Most patients were warned to stay home, and all new cases were kept out of dispensaries. This may have accounted for some reduction in the incidence. However, in some clinics where such precautions were not observed, the disease spread but very little into the population.

Also, in various dispensaries and clinics with which we came in contact there was practically no involvement of professional personnel.

Clinical characteristics

The disease as observed in our patients was characterized by the initial development of an acute conjunctivitis with edema of the lids and conjunctiva (fig. 1). Except for

* From the Francis I. Proctor Foundation for Research in Ophthalmology and the Department of Ophthalmology, University of California School of Medicine.

* Hogan, M. J., and Crawford, J. W.: Epidemic keratoconjunctivitis. Am. J. Ophth., **25**:1059-1078 (Sept.) 1942.

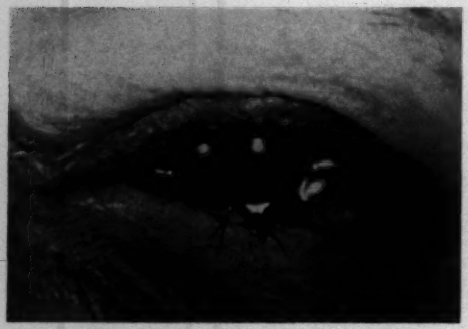

Fig. 1 (Hogan). Lid and conjunctival edema.

Fig. 2 (Hogan). Epidemic keratoconjunctivitis chemosis.

Fig. 3 (Hogan). Chemosis in the early acute stage.

tearing there was no discharge. The edema of the conjunctiva and eyelids rapidly increased (figs. 2 and 3). In 25 percent of the cases the disease was bilateral, and there was a tendency for the second eye to be affected at a later date and to a lesser degree.

Within 36 to 48 hours the regional lymphatics became involved and in 75 percent of the cases preauricular, angular, submaxillary, and cervical lymph-node enlargement and tenderness appeared. Early examination of the conjunctiva itself showed a slight hyperemia with glassy edema. Later the hyperemia became more intense and in slightly less than 50 percent of the cases follicles appeared on the lid conjunctiva (fig. 4). Occasional cases showed petechias and two cases had severe ecchymosis of the conjunctiva and lids. Seventeen cases showed pseudo- or true membranes (figs. 5 and 6) on the conjunctiva of the lid and fornix. The conjunctivitis lasted an average of 13 days in this group of patients.

Ninety-two of the 125 patients developed the typical keratitis within two to eight days after onset of the conjunctivitis and in 23

Fig. 4 (Hogan). Follicular enlargement in the lower conjunctiva.

Fig. 5 (Hogan). Pseudomembrane in early epidemic keratoconjunctivitis.

Fig. 6 (Hogan). Membrane formation in the upper lid.

Fig. 7 (Hogan). Corneal infiltrates.

of these it became bilateral. The onset of keratitis was characterized by the development of pain and severe photophobia. Several types of lesions were seen in the cornea: (1) Nonspecific superficial punctuate erosions, which stained; (2) the typical macular lesions which are diagnostic. They were 0.5 to 1.5 mm. in size, round or oval, just beneath Bowman's membrane (figs. 7 and 8), more centrally located and varied in number between 10 and 100. It was characteristic that these lesions did not stain, ulcerate, or become vascularized. Corneal sensation was normal in all cases. In 17 patients deeper stromal lesions were seen, and, of these, six had a secondary iritis. Several patients developed disciform lesions in the corneal stroma.

The corneal lesions outlasted the conjunctivitis by a considerable period, the eyes remaining photophobic and slightly congested for up to a month. In most patients gradual fading of the infiltrates occurred during a period of two to three months and vision became normal. Those patients with deeper lesions or disciform infiltrates had 20/40 to 20/50 vision up to four to six months afterward but an eventual return to normal occurred in all cases.

A number of cases were followed for a year, and a few were seen as late as 10 years after the infection. We have not seen a recurrence in any of these cases.

Fig. 8 (Hogan). Corneal infiltrates.

Cultural studies and cytology

Routine cultures from the conjunctiva during the acute phase of the conjunctivitis were invariably negative, and scrapings showed a lymphocytic type of exudate. Conjunctival washings and scrapings were inoculated intracerebrally and intraperitoneally into mice from many patients, without effect. Efforts to reproduce the disease in the eyes of mice, guinea pigs, rabbits, dogs, and monkeys failed.

Therapy

Attempts to control the disease with various therapeutic agents were not successful.

SUMMARY

1. The San Francisco Bay area outbreak of epidemic keratoconjunctivitis appeared primarily in shipyard workers, and seemed to be spread by contaminated hands and dispensary equipment and solutions rather than by occupational equipment.

2. The frequency of spread to professional personnel and to families of infected workers was very low.

3. One hundred twenty-five cases were studied and the following findings were most important:

a. An acute conjunctivitis with lid and mucosal edema, and *without* purulent discharge. In 25 percent the disease was bilateral.

b. Regional lymphadenitis occurred in 75 percent of patients.

c. Fifty percent of patients had a follicular conjunctivitis.

d. Thirteen percent of patients had true or pseudomembranes on the conjunctiva.

e. Ninety-two of the 125 patients developed the typical keratitis. In 17 patients disciform or other stromal involvement was seen. In 23 patients the corneal lesions became bilateral.

4. Negative cultures and a lymphocytic type of exudate were found.

KERATOCONJUNCTIVITIS*

THE CLINICAL CHARACTERISTICS OF THE NEW YORK EPIDEMIC, 1943-1946

ALSON E. BRALEY, M.D.
Iowa City, Iowa

Sporadic cases of epidemic keratoconjunctivitis began appearing in the New York area in the spring of 1942. The ophthalmologists in the area had been alerted to a disease called shipyard conjunctivitis or California conjunctivitis by our friends on the Pacific Coast. The early cases of epidemic keratoconjunctivitis were not recognized because they seemed to be comparatively mild. But in reviewing some of these early cases, it was possible to make the diagnosis.

The clinical disease increased in severity as the number of cases increased in number. In June, 1942,[1] Mr. R. H. appeared at the Vanderbilt Clinic with a history that the previous night he had felt a sudden sharp pain in his right eye. This was associated with a foreign-body sensation. He was unable to find a foreign body but the sensation continued through the following morning. The following morning there was considerable edema of the upper lid and it was difficult for him to open his eye. There was moderate to severe pain, and discomfort to his eye, especially on rotation of the eyeball. Vision was normal. When he presented himself at the clinic, there was marked edema of the upper lid and moderate chemosis. The conjunctiva was smooth but the blood vessels were distended. There was edema of the semilunar fold and caruncle.

Scrapings from the conjunctiva showed epithelial cells with numerous mononuclear leukocytes. No polymorphonuclear leukocytes could be found.

The second day the edema had increased considerably and there began to appear small follicles on the lower palpebral conjunctiva and a few follicles at the upper margins of the tarsus of the upper lid. The edema of the caruncle and semilunar fold increased and

* From the Department of Ophthalmology, University of Iowa College of Medicine.

there was a slightly tender palpable preauricular lymph node.

During the next five days the clinical findings gradually increased until there was an advanced follicular conjunctivitis. Photophobia was not particularly troublesome. On the fifth day the edema began to decrease and the palpable preauricular node began to be less evident and not tender. Submental and submaxillary lymph nodes were then present.

On the sixth day of the disease there was a slight photophobia. Examination with a biomicroscope demonstrated very fine subepithelial opacities. There was no staining of the cornea with fluorescein. The edema of the left eye had increased; however, the edema of the right eye had decreased.

On the eighth day, the conjunctivitis in the right eye had improved somewhat but there were numerous 0.5-mm. whitish opacities in the cornea. These opacities were just beneath the epithelium and seemed to raise the epithelium in some areas. They did not stain with fluorescein. Approximately 30 opacities were counted in the cornea. The opacities were circular and composed of numerous fine dots. The vision in this eye was decreased to 20/70.

The acute conjunctivitis lasted four weeks in each eye. It improved slowly during the last weeks. The visual disturbance and the corneal opacities persisted. Six months later, the vision in the right eye had improved to 20/30 and numerous subepithelial corneal opacities were still present. In the left eye which had been involved secondarily, the vision was not disturbed. One year later, the right cornea showed eight opacities while the left eye had two. The vision in the right eye was still 20/30 and there was mild photophobia in artificial light.

The number of cases seen gradually increased and they seemed to increase somewhat in severity, so that during the winter of 1942 and early 1943, the disease appeared to be more hyperacute and a pseudomembrane would frequently appear on the third to fifth day of the disease. A pseudomembrane was present in 67 percent of the cases that I saw. These pseudomembranes were light yellow in color and contained many mononuclear cells and degenerating epithelial cells.

In late 1943,[2] I summarized the clinical appearance of the disease into four stages.

Stage 1 is the acute phase in which the primary symptoms are those of foreign body sensation, with burning and marked tearing. There develops edema of the upper lid with chemosis and edema of the semilunar fold and caruncle. There is hyperemia of the conjunctival blood vessels. Ocular rotations may be painful or associated with discomfort.

Stage 2. The disease enters the second stage in approximately 48 hours. This stage is characterized by the development of large follicles in the conjunctiva, and sometimes many subconjunctival infiltrates. Preauricular lymph adenopathy is usually present during this time. The follicles gradually increase and in many instances a thick pseudomembrane appears on the palpebral and at times on the bulbar conjunctiva. This is usually present by about the fifth day.

Stage 3. The third stage of the disease is the continued follicular conjunctivitis associated with beginning corneal changes. These corneal changes produce photophobia and blurring of vision. At times, the blurring of vision is quite marked, particularly when there are opacities on the anterior surface of the cornea and occasionally in the stroma. These opacities usually begin on about the seventh to the 10th day of the disease although they may not be apparent for several days later.

Stage 4. The fourth stage of the disease is characterized by a disappearance of the conjunctivitis and a continuation of the keratitis. This keratitis may last for many months to years.

The epidemic seemed to burn itself out during the early part of 1944, and only sporadic cases of epidemic keratoconjunctivitis

were seen at the St. Albans Naval Hospital in New York City. However, in the winter and early spring of 1946, a small but interesting epidemic developed in an area near the Vanderbilt Clinic. Quite a number of patients with the disease were seen in the professional personnel of the hospital and individuals from the surrounding area.

During this period, the clinical disease was characterized by much more severe corneal findings that conjunctival findings. The conjunctival disease was less acute. There was moderate to slight edema of the conjunctiva and of the upper lid. However, the follicular conjunctivitis was present.

After the follicular conjunctivitis had been present for approximately 10 days to two weeks, the severe keratitis would develop. The corneal opacities would first appear just beneath Bowman's membrane. These would increase in number and severity and small opacities could be found in the deeper stroma. Fine deposits were frequently present on the posterior surface of the cornea and Tyndall's phenomenon was present in the anterior chamber. A mild to moderately severe iritis was often encountered.

During the 1942-1943 epidemic, the corneal opacities would usually disappear within a few months to two years but, of individuals who had the disease during the 1946 epidemic, some of them still have corneal opacities. One of these later patients, Dr. J. W., was examined in April, 1956. With a simple flashlight, it was possible to see three white leukoma in the pupillary area of the right eye. The vision in this eye was a poor 20/20.

SUMMARY

The cases of epidemic keratoconjunctivitis seen in New York during the early part of 1942 were characterized by a hyperacute conjunctivitis which became a follicular conjunctivitis associated with a preauricular lymph adenopathy. The early cases seen were mild and the severity of the disease increased as the number of cases increased. Corneal opacities were present in 92 percent of the cases in which diagnosis was made. During the 1946 epidemic, the conjunctivitis was less marked and the corneal disease was more evident.

University Hospitals.

REFERENCES

1. Braley, A. E.: Epidemic keratoconjunctivitis. Med. Clinics North America, Nov., 1943, pp. 1641-1651.
2. ———: Epidemic keratoconjunctivitis. Tr. Am. Acad. Ophth., Jan.-Feb., 1944, pp. 153-174.

EPIDEMIC KERATOCONJUNCTIVITIS IN JAPAN AND THE ORIENT*

CHIE TANAKA,† M.D.
Tokyo, Japan

INCIDENCE

The first report on epidemic keratoconjunctivitis (EKC) in Japan was made by Inoue[1] in 1894. In 1897, Asayama[2] reported 10 cases of this condition. However, epidemic keratoconjunctivitis seems to have been relatively uncommon before the year 1937. From that year on, the incidence increased rapidly and frequent epidemics occurred, especially from sources of contamination in swimming pools and physicians' offices.

Examination of statistics found in the Japanese literature shows that the incidence

* From the Department of Ophthalmology, Tokyo Women's Medical College.
† Research associate, Ophthalmology Branch, National Institutes of Health, Bethesda, Maryland.

has rarely fallen to less than one percent of clinic patients during the past 20 years.[3-7]

During a six-month period in 1941, Okamura and Mitsui[5] observed 33 cases of epidemic keratoconjunctivitis among some 4,000 out-patients at the Tokyo Imperial University Clinic. In the same year, Aoki and Kasahara[6] observed 72 cases among some 3,500 out-patients at a hospital in Tokyo. Over a two-year period between 1948 and 1949, Mitsui and Tanaka[7] observed 215 cases among about 10,000 out-patients at Kumamoto University Clinic.

Epidemic keratoconjunctivitis seems to be common throughout the Orient, though information about it is limited. Sources of information include those of Wright[8] and Kirwan[9] in India, Kumano[10] in Formosa, and Oishi[11] in Manchuria.

During the past 20 years, epidemic keratoconjunctivitis has been observed sporadically in Japan in all seasons of the year, with occasional outbreaks of epidemics.

Human inoculations

Epidemic keratoconjunctivitis inoculation of humans was first done by Wright[8] (1930) in India. Kirwan[9] performed the same procedure in 1935. The first inoculation in Japan was made by Aoki[12] in 1941. His objectives were to confirm the infectious nature of this condition, as well as to follow the incubation period and whole clinical course of the infection. The result of inoculation was positive in 12 of 14 cases, including two subinoculations. The incubation period averaged five days. In all of the cases, the disease infected the second eye in the course of a week. Subepithelial punctate keratitis appeared in 16 of the 24 eyes, eight to 11 days after the onset of conjunctivitis. Aoki also described the histology of this condition.

A second series of inoculations was performed by Mitsui[13] in 1948. His purpose was to follow the nature of membranous or pseudomembranous conjunctivitis in infants, a condition which is widespread in Japan and very often associated with definite systemic symptoms. This problem will be discussed later.

A third series of inoculations was performed by Tanaka[14,15] in 1950 and 1951. Her objective was twofold. The first was to determine the nature of membranous or pseudomembranous conjunctivitis in infants with systemic symptoms in which Koch-Weeks bacilli were demonstrated by smear examinations. The second was to follow the immunity of epidemic keratoconjunctivitis. These two problems will be discussed in separate sections.

An interesting but still unpublished study on inoculation is that of Hinokuma.[16] He observed an exceptional case in which typical subepithelial punctate keratitis without accompanying conjunctivitis resulted from the inoculation. It is very interesting to compare the result in this case with that obtained by Thygeson[17] who observed punctate keratitis without conjunctivitis in a woman whose husband, a physician, had been suffering from typical epidemic keratoconjunctivitis.

Clinical features

Clinical symptoms of epidemic keratoconjunctivitis in Japanese adults are much the same as those described in reports from Europe and the United States. There is acute follicular or, less frequently, pseudomembranous conjunctivitis of about one month's duration. Monocytes predominate in conjunctival smears and the bacteriology is essentially negative. A protracted incubation period is suggested by the wide interval between the onset of the disease in the first and the second eye in those cases where the condition is bilateral. A preauricular adenopathy is very often associated with epidemic keratoconjunctivitis.

The incidence of subepithelial punctate keratitis varies greatly from one epidemic to another and this may be at least partially due to the difference in the age[23] of the sufferers in a given epidemic. A review of the literature shows that the incidence of keratitis is in the range of 35 to 60 percent

(Otsuka,[18] Mitsui and Tanaka,[7] and Mori[19]).

Systemic symptoms are rarely found in adults and, when present, are very slight.

Recently, Mitsui[20] observed several cases of epidemic keratoconjunctivitis among Europeans and Americans during an epidemic in Kumamoto. When he compared these cases with those of Japanese subjects, he received the impression that keratitis is apt to be more severe in the Caucasian race than in Japanese.

The infantile form of epidemic keratoconjunctivitis was first described by Mitsui and Kudo[21] in 1944, but was known to have been widespread in Japan before that time. The clinical picture was very different from that of epidemic keratoconjunctivitis in adults, and therefore, the infantile form was regarded as a different disease.

The infantile form of epidemic keratoconjunctivitis is a pseudomembranous or membranous conjunctivitis usually affecting infants less than two years old and, less frequently, young children up to five years of age. It is very often (about half of the cases) accompanied by definite systemic symptoms such as high fever, angina, pharyngitis, otitis media, diarrhea, and vomiting. Mitsui stated that many of the patients had first visited pediatricians because of the systemic symptoms, and from that source they had been referred to him.

It is interesting to note that an association with keratitis is extremely exceptional in the infantile form. Preauricular adenopathy is also absent as a rule in infants under two years of age.

In their first report, Mitsui and Kudo merely suggested the possibility that this particular type of pseudomembranous conjunctivitis was an "infantile form of epidemic keratoconjunctivitis." By experimental inoculations in humans, however, Mitsui[13] later demonstrated that the material from "infantile epidemic keratoconjunctivitis" could cause typical epidemic keratoconjunctivitis in the adult, and vice versa. The outline of this study was introduced to readers of English by Mitsui, Tanaka, and Yamashita.[23] Their findings were confirmed by Aoki[22] and Mori.[19]

Most recently, Mitsui[20] obtained acute and convalescent sera from two typical cases of "infantile epidemic keratoconjunctivitis" (according to his criterion) and sent samples to Jawetz in San Francisco. Jawetz[24] demonstrated a considerable rise of neutralizing antibodies against adenovirus type 8 in the convalescent serum.

Mode of transmission

The mode of transmission is very often obscure in endemic or sporadic cases except where transmission in families is established.

Epidemics are usually mediated by sources of contamination in swimming pools and physicians' offices, but there seems to be no report of epidemics in industrial plants such as shipyards.

In 1947, Otsuka[18] reported an epidemic of 46 cases which originated in a swimming pool in Tokyo. In 1955, Mitsui[20] studied an epidemic in a junior high school in Kumamoto. Nearly 200 students who bathed in the swimming pool of the school suffered from this condition over a four-week period. He also observed two sizable epidemics in the eye clinic at Kumamoto University. During the first epidemic the source of infection remained obscure. The second epidemic was definitely mediated by a topical anesthetic solution for tonometry. More than 60 cases were infected through these two iatrogenic sources.

Transmission through family contact is commonly found in Japan. Thirteen of the 72 cases observed by Aoki and Kasahara[6] were so transmitted. Their study included a family in which seven members were successively infected, probably from other members of the same family. Mori[19] reported six cases of family infection among 17 cases studied.

Immunity

The result of the inoculation of epidemic

keratoconjunctivitis virus in humans was not always positive, even though cases without a history of acute conjunctivitis were selected as the volunteers (Aoki,[12] Mitsui,[13] and Tanaka[14,15]). Nonsusceptible cases amounted to about 15 percent according to Aoki and about 30 percent according to Mitsui.

Tanaka repeated inoculations in such negative cases with materials obtained from different donors after a certain interval. The result was consistently negative. She was able to demonstrate the active virulence of the inoculum by inoculating the same material into another volunteer. She found three out of a total of eight volunteers to be nonsusceptible.

Mixed infection

Later, Tanaka[14] demonstrated by an inoculation experiment that certain cases represented a mixed infection of epidemic keratoconjunctivitis and Koch-Weeks conjunctivitis. She treated one such case with $AgNO_3$ and, after the disappearance of the bacilli, inoculated conjunctival secretions from this patient into a normal adult eye. This resulted in the onset of a typical epidemic keratoconjunctivitis picture with definite keratitis.

Since the discovery of antibiotics, there has been a rapid decrease in bacterial conjunctivitis in Japan. Mixed infections of epidemic keratoconjunctivitis and bacterial conjunctivitis appear to have become uncommon.

The combination of epidemic keratoconjunctivitis and herpetic keratitis has not yet been reported in Japan.

Summary

1. Epidemic keratoconjunctivitis seems to have been uncommon in Japan before 1937, but from that year on the incidence increased. Statistically, epidemic keratoconjunctivitis patients constitute between one and two percent of clinic patients.

2. By inoculation of epidemic keratoconjunctivitis into humans, it was found that the incubation period averaged five days; that the disease infected the second eye in the course of a week; and that keratitis appeared in about 60 percent of the cases, eight to 11 days after the onset of conjunctivitis.

3. Epidemic keratoconjunctivitis in infants is very different from that in adults. It is a pseudomembranous conjunctivitis, and is frequently accompanied by definite systemic symptoms but rarely associated with keratitis or preauricular adenopathy.

4. Epidemic keratoconjunctivitis is endemic or sporadic in occurrence. Transmission is by contact with sources of contamination such as swimming pools and physicians' offices, or by familial contact.

5. The result of epidemic keratoconjunctivitis inoculation in humans was not always positive. Between 15 and 30 percent were found to be nonsusceptible.

6. With the advent of antibiotics, mixed epidemic keratoconjunctivitis and bacterial infections have become uncommon.

References

1. Inoue, M.: Superficial punctate keratitis. Ganka Zasshi., **1**:273, 1894.
2. Asayama, I.: The histology of superficial punctate keratitis. Acta. Soc. Ophth., Japan, **1**:427, 1897.
3. Oguchi, T.: Superficial punctate keratitis and nummular keratitis. Chuo Ganka Iho, **27**:62, 1935.
4. Ogino, R.: Superficial punctate keratitis. Chuo Ganks Iho, **32**:72, 1940.
5. Okamura, K., and Mitsui, Y.: Diagnostic value of Prowazek's inclusion bodies in trachoma. Acta Soc. Ophth. Japan, **45**:108, 1941.
6. Aoki, H., and Kasahara, C.: Epidemic keratoconjunctivitis. Acta Soc. Ophth. Japan, **45**:573, 1941.
7. Mitsui, Y., and Tanaka, C.: Constitution and conjunctivitis, first report. Acta Soc. Ophth. Japan, **54**: 346, 1950.
8. Wright, R. E.: Superficial punctate keratitis. Brit. J. Ophth., **14**:257 and 595, 1930.
9. Kirwan, E. W.: Epidemic superficial punctate keratitis in Bangal. Folia Ophth. Orient., **1**:345, 1935.
10. Kumano, S.: Superficial punctate keratitis. Acta Soc. Ophth. Japan, **43**:1747, 1939.

11. Oishi, S.: Inclusion conjunctivitis and trachoma with special reference to cytology of the smear. Acta Soc. Ophth. Japan, **42**:538, 1938.
12. Aoki, H.: Some observations on epidemic keratoconjunctivitis. Acta Soc. Ophth. Japan, **45**:827, 1941.
13. Mitsui, Y.: Nature of membranous conjunctivitis in infants. Acta Soc. Ophth. Japan, **52**:75, 1948.
14. Tanaka, C.: Pseudomembranous conjunctivitis in infants with positive findings of Koch-Weeks bacilli. Ganka Rinsho Iho, **44**:109, 1950.
15. ———: Immunity of epidemic keratoconjunctivitis. Ganka Rinsho Iho, **45**:456, 1951.
16. Hinokuma, S.: Personal communication.
17. Thygeson, P.: Personal communication.
18. Otsuka, J.: Swimming pool conjunctivitis and its relation to epidemic keratoconjunctivitis. Rinsho Ganka, **1**:92, 1947.
19. Mori, N.: Symptomatology of epidemic keratoconjunctivitis. Acta Soc. Ophth. Japan, **47**:1264, 1953.
20. Mitsui, Y.: Personal communication.
21. Mitsui, Y., and Kudo, T.: Membranous conjunctivitis in infants with systemic symptoms. Sogo Ganka Zasshi, **39**:182, 1944.
22. Aoki, H.: An experimental study of epidemic keratoconjunctivitis. Acta Soc. Ophth. Japan, **53**: 146, 1949.
23. Mitsui, Y., Tanaka, C., and Yamashita, K.: Change in the constitution with age and its influence on the clinical symptoms of conjunctivitis. Am. J. Ophth., **39**:540, 1955.
24. Jawetz, E.: Personal communication.

EPIDEMIC KERATOCONJUNCTIVITIS IN ITALY*

Some contributions to its clinical aspects, epidemiology, and etiology

G. B. Bietti, M.D., and F. Bruna, M.D.
Rome, Italy

Clinical and epidemiologic observations

Epidemic keratoconjunctivitis was observed for the first time in Italy (1942) in the Eye Department of the University of Sassari (Sardinia[19]) by one of us (Bietti) and shortly afterward in Rome.[9] These findings coincided with the arrival of German troops who possibly carried the disease with them, since epidemic keratoconjunctivitis was very prevalent in Germany at that time.

Our observations in Sassari (already published by Pasca[19]) referred to 1,094 cases occurring mostly during the months of September (192), October (252), November (173), and December (71), 1943. The disease showed a familial incidence in 97 cases (36 families). It was unilateral in 20 percent of the patients; corneal involvement was observed in about 38 percent of the cases. Among 274 patients under 10 years of age, 49 had corneal complications, while in 245 subjects from 10 to 20 years, 124 had corneal involvement.

Gradually in the following years epidemic keratoconjunctivitis has become more or less common in Italy, and cases of it have been observed in practically every region.[1-3, 5-12, 14, 17-25] Bietti saw several cases in Pavia and Parma.[8]

The questioning of colleagues of the principal centers of the country has given the results which are summarized in Table 1 and the map (fig. 1) which show the diffusion of epidemic keratoconjunctivitis in Italy.

Our recent observations concern the cases seen in the out-patient department of the University Eye Clinic of Rome. A total of 1,093 patients, having a typical epidemic keratoconjunctivitis or only the conjunctival

* From the Department of Ophthalmology, University of Rome. (Director: Prof. G. B. Bietti.)

(See Table 1 on folded insert.)

Fig 1 (Bietti and Bruna). Chief epidemic keratoconjunctivitis areas in Italy during the years 1943 to 1956.

aspects characteristic of the disease, but without corneal involvement, was observed from October, 1942, to July 1, 1956. The microscopic examination, as well as the clinical and epidemiologic patterns, permitted differentiation of the purely conjunctival cases from other follicular manifestations of the conjunctiva, mainly inclusion and Béal's conjunctivitis, and to consider them as incomplete cases of epidemic keratoconjunctivitis. It must be noted, however, that, in early cases of conjunctivitis, corneal involvement probably occurred after our examination in a later stage and was not observed by us. Of the 1,093 cases, 367 were unilateral and 726 bilateral.

The age distribution of the disease, as well as the incidence of the corneal involvement in the different months and years, is shown in Tables 2 and 3.

From our data, as well from those of other Italian ophthalmologists, the following conclusions may be drawn regarding the epidemiology of epidemic keratoconjunctivitis in Italy:

1. The morbidity index of the disease is generally low, since epidemic keratoconjunctivitis has almost never reached the aspect of a serious epidemic; the only true epidemics of epidemic keratoconjunctivitis have been recorded, as already mentioned, in Sassari,[19] 1943-44 (1,094 cases), and in Genoa[20-22] in 1953-54 (where the disease occurred among the workers of the Ansaldo Shipyards).

2. Regarding geographic distribution, as

TABLE 2

DISTRIBUTION OF EPIDEMIC KERATOCONJUNCTIVITIS IN THE OUT-PATIENTS OF THE UNIVERSITY EYE CLINIC OF ROME FROM 1942 TO 1956, AND INCIDENCE OF CORNEAL INVOLVEMENT

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Cases	Yearly Percent	Corneal Lesions	Percent of Corneal Involvement
1942										1	1		2	0.016	2	
1943		1				2	1						4	0.034	4	
1944			1				2						3	0.037	3	
1945						2	12	5		3	6		28	0.22	7	25
1946			3	3		11	8	6		14	11	11	67	0.52	27	40.02
1947	16	19	41	28	17	48	7		16	13	14	20	229	1.62	69	30.1
1948	27	9	6	16	17	27	8					10	120	1.89	17	14.1
1949	11	15	7	15	12	11	15	9	25	17	10	8	155	1.16	20	12.9
1950	1	18	18	11	10	11	8	11	5	5	2	4	104	0.88	21	20.1
1951	1		1	1	8	3	2	4		2	2	6	30	0.023	5	16.6
1952	6	9	5	2	5		7	5	7	4			50	0.38	3	6.0
1953					4	5	5	2	1	3	3	6	29	0.24	2	6.8
1954	4	1	10	13	16	13	11	9	10	10	10	7	114	0.93	8	7.0
1955	9	8	7	18	18	14	5	5	11	4	2	4	105	0.82	11	10.4
1956	6	5	5	11	14	12							53	0.82	7	13.2
	81	85	104	118	121	159	91	56	75	66	61	76	1093		206	

The figures of 1956 refer to the first half year.

TABLE 3

AGE DISTRIBUTION OF EPIDEMIC KERATOCONJUNCTIVITIS WITHOUT AND WITH CORNEAL INVOLVEMENT IN THE OUT-PATIENTS OF THE UNIVERSITY EYE CLINIC OF ROME FROM 1942 TO 1956

Age (yr.)	Conjunctivitis Cases	Cases with Corneal Lesions	Corneal Lesions %
Below 5	19	1	5.2
5–10	39	1	2.5
11–20	149	27	18.1
21–30	255	67	26.2
31–40	237	42	17.7
41–50	207	45	21.7
51–60	120	18	15.0
61–70	51	3	5.8
71–80	13	2	15.3

may be seen from the annexed map, South Italy seems to have been least attacked by the disease.

3. Independent from the latitude, no month of the year has been spared by the disease, which has, however, a higher incidence during the spring and the autumn seasons.

4. No difference in the incidence of epidemic keratoconjunctivitis has been observed between males and females, except in industrial centers where males are generally affected in greater numbers.

5. Among the most common types of contamination the following have been found: (a) family; (b) communities, such as schools, orphanages, religious groups; (c) contact in dispensaries or hospitals due to eyedroppers, surgical instruments, tonometers, and so forth. Also the epidemics observed among factory workers have been attributed in the big factories to contamination in local dispensaries (in Turin Fiat factories; Genoa and Leghorn, shipyards), the incidence of the disease paralleling the number of workers attending the factory's dispensaries.

6. Our observations permit the following conclusions regarding the clinical picture of epidemic keratoconjunctivitis in our area:

a. The conjunctival involvement may differ greatly from case to case: sometimes it is extremely mild, while in other cases it appears particularly severe with marked secretion, pseudomembrane formation, considerable hypertrophy, resembling thus the aspect of an acute trachoma, or of a swimming-pool conjunctivitis. In some cases the conjunctivitis has been even overlooked by the patients, who came to us complaining only about visual troubles due to corneal changes.

The duration of the conjunctivitis may differ (one to four weeks or more) even for the same area; no relationship to year or season of occurrence has been found.

b. Corneal involvement may occur in different incidence in different years. As shown in the tables, confirming thus our previous observations in Sassari, adults are more affected by corneal complications than children below the age of 10 years.

Peculiar types of corneal involvement have often been encountered in addition to the characteristic one: microphlyctenular eruptions at the limbus; marginal infiltration and ulceration; bullous keratitis; filamentous keratitis; superficial ulcerated keratitis of the dendritic type (three cases); deep keratitis with a disciform aspect (two cases). All these changes were observed coincident with or after the appearance of the typical round infiltrations of the disease.

The duration of the corneal changes is sometimes extremely long: two to three years from their occurrence. According to our experience, however, they finally almost always disappear leaving no scars. In one case, however, we saw definite evidence of previous corneal changes 10 years after an epidemic keratoconjunctivitis outbreak.

c. Sometimes a particularly severe episcleral reaction has been observed. A mild iritis, with or without corneal involvement, has been encountered 23 times; in one of these cases spontaneous hemorrhage occurred in the anterior chamber.

d. In some cases herpetic extraocular lesions have been observed at different times during the course of the epidemic keratoconjunctivitis.

It is to be noted that the epidemics of Sassari in 1943 were observed during a

period characterized by a considerable increase (about five times) of herpetic keratitis, in association with the higher incidence of malaria attacks due to war conditions in Sardinia which were frequently accompanied by herpetic eruptions. On the other hand, however, epidemic keratoconjunctivitis was of unusually low incidence during the winter 1953-54, when herpetic keratitis was particularly frequent in Rome and was associated with a diffuse epidemic of influenza (type A).

e. As to the therapy of epidemic keratoconjunctivitis, the following remarks may be made:

1. The use of antibiotics, especially Chloromycetin, aureomycin, terramycin, has found large acceptance by ophthalmologists but, according to our experience in 200 consecutive cases with untreated control groups, their efficacy has not been proved.

2. We have, therefore, chiefly been using silver preparations (argyrol drops and painting of the everted conjunctiva with silver nitrate, two percent) to act chemically and mechanically (desquamation) on the virus, which is apparently resistant to antibiotics.

3. Cortisone does not give constant results; some colleagues stress a regression of the corneal lesions, especially during the phase of ulceration. We generally refrain from the use of cortisone in the first stage of the disease, especially when the conjunctivitis is still active, and prescribe it later as prophylaxis against the corneal changes.

Etiologic investigations

The findings recently published by Jawetz, Thygeson, and co-workers,[15,16] regarding the importance of the adenovirus type 8 as an etiologic agent of epidemic keratoconjunctivitis, stimulated us to perform some investigations in order to support these views.

Material and techniques

For our researches (cultivation, complement fixation and neutralization test, inoculation) an adenovirus type 8 strain, kindly supplied by Dr. Jawetz and cultivated by us in HeLa cells with the assistance of Dr. Balducci of the Institute of Public Health in Rome, has been used.

The following *culture media* have been utilized:

	Percent
a. Growth medium:	
human serum	30
lacto-albumin hydrolysate	5-10
Hanks solution	60
b. Maintenance medium:	
synthetic medium 199	92
rabbit serum	5
chicken serum	3

Attempts to cultivate the virus have been made also in human liver cells (Chang's strain) and in rabbit kidney cultures.

For the cultivation in liver cells the media have been prepared as follows:

	Percent
a. Growth medium:	
human serum	5
horse serum	25
lacto-albumin hydrolysate	5
Hanks solution	65
b. Maintenance medium:	
medium 199	95
horse serum	5

For the cultivation in rabbit kidneys:

	Percent
a. Growth medium:	
calf serum	10
lacto-albumin hydrolysate	5
Hanks solution	85
b. Maintenance medium	
calf serum	5
lacto-albumin hydrolysate	5
Hanks solution	90

The HeLa and liver cells have been cultivated in flasks and then transferred into test tubes with trypsinization (trypsin Difco 1:250, diluted 0.25 percent in Hanks solution). The cultures of the test tubes were used within two to four days from their preparation, after having been washed three times in Hanks solution.

The cultures in rabbit kidney were prepared by obtaining a suspension of the kidney through trypsinization.

Complement fixation test has been performed following the "drop" microtechnique, on perspex plates, according to Fulton and Dumbell[13] with the modification of Balducci.[4] As antigen the fluid of HeLa culture of an adenovirus type 3 strain was used; the fluid was collected when the cytopathic degeneration was almost complete, then frozen and

thawed three times and centrifuged at 600 rpm for 10 minutes; the supernatant was employed as antigen, the optimum unit being obtained by titration with positive human serum.

Neutralization tests have been performed in HeLa cells cultures. The sera for the test have been diluted 1:4 and then inactivated. Further dilutions have been made with Hanks solution in a volume of 0.60 ml. The same amount of undiluted virus suspension was added. The mixture was kept one hour at room temperature and then two test tubes were inoculated with 0.5 ml. of the mixture.

Results

Regarding the biologic behavior of the adenovirus type 8 in HeLa cell cultures we, too, in accordance with the observation of Jawetz and co-workers, have noted that the cytopathic effect progresses very slowly, even if, exceptionally, it can be observed after two or three days.

In addition to this occurrence (which has been particularly embarrassing for us, owing to the short time at our disposal before the date of epidemic keratoconjunctivitis symposium in San Francisco) the virus showed a particularly slow increase of the infectious titer; it actually never exceeded the figure of $10^{-1.5}$.

As already mentioned, attempts have also been made to cultivate the virus in human liver cells and in rabbit kidney but, in both instances, the results have been, for our purpose, practically negative. In effect, the liver cell cultures, even if surely sensitive, require, as the HeLa cell cultures and perhaps more, a long period to show growth. On the other hand, the rabbit kidney cultures do not seem to reach a specific cytopathic effect; nor has it been possible to reisolate the virus in HeLa cell cultures, even if some apparent cytopathic effect was observed after about 48 hours but without clear progression in the following days.

The complement fixation and neutralization tests have been performed on sera of patients who had shown epidemic keratoconjunctivitis during the last 18 months or were still suffering from the disease. They came from the provinces of Rome, Grosseto (central Italy), Bologna, Ferrara, and Piacenza (northern Italy).

From Table 4 it may be seen that the complement fixation test yielded positive results in 35 out of 62 cases. In four additional cases (table 6) the result was always positive, bringing the total of positive reactions to 58.7 percent.

The neutralization test in HeLa cell culture gave definite results in only 19 tubes out of 66 sera, owing to the short time at our disposal.

In spite of the limited number of patients who could be analyzed, the results obtained appear of importance because of the high positive titer for adenovirus type 8 in the majority of these cases.

From four of our patients, paired sera could be obtained; the former during the acute phase, the latter two weeks after the onset of the conjunctival changes. The results, seen in Table 6, are highly significant.

In addition to the aforementioned data the results previously obtained by Jawetz and co-workers from serologic examinations on other sera sent by one of us (Bietti) from Italy should be remembered. Personal com-

TABLE 4

Results of the complement-fixation test for the adenoviruses in epidemic keratoconjunctivitis patients from different Italian provinces

Dilution	<1:4	1:4	1:8	1:16	1:32	>1:32
No. of sera	27	11	13	5	5	1

TABLE 5

RESULTS OF THE NEUTRALIZATION TEST FOR THE ADENOVIRUS TYPE 8 IN 19 EPIDEMIC KERATOCONJUNCTIVITIS PATIENTS FROM DIFFERENT ITALIAN PROVINCES

Dilution	<1:32	1:32	1:64	1:128	>1:128
No. of sera	4	4	4	2	5

TABLE 6

RESULTS OF THE COMPLEMENT-FIXATION AND NEUTRALIZATION TESTS IN PAIRED SERA (IN THE ACUTE PHASE AND 15 DAYS AFTER ONSET OF EPIDEMIC KERATOCONJUNCTIVITIS) OF FOUR PATIENTS

Patient	Complement Fixation		Neutralization	
	1st Test	2nd Test	1st Test	2nd Test
U. F. (Rome)	<1:4	1:16	1:64	1:512
P. G. (Rome)	1:8	1:32	1:256	>1:512
A. G. (Rome)	1:8	1:32	1:32	>1:512
P. S. (Rome)	1:6	anticompl.	1:32	>1:512

munications from Thygeson informed us of the isolation of the adenovirus type 8 strain in Jawetz's laboratory.

Thirteen sera belonging to patients from the Como Lake area in northern Italy (where our co-worker, Giardini, had recently observed a typical epidemic keratoconjunctivitis) and 24 control sera (from Como and Parma) were thus examined. The sera of the 13 patients with epidemic keratoconjunctivitis constantly showed the presence of neutralizing antibodies: five at a titer of 1:20; four at 1:4; four at 1:50. The neutralization tests performed on the 24 control sera always gave negative results; only one case was positive at the 1:10 titer.[15]

By adding these results with those more recently obtained by us, it appears that, among 36 patients from Italy who had epidemic keratoconjunctivitis during a period of 18 months or less before the serologic examination, 32 showed positive neutralization tests for the adenovirus type 8.

We feel that these findings further support the importance of the role of the adenovirus type 8 in the etiology of the epidemic keratoconjunctivitis.

Attempts have also been made by us to transmit epidemic keratoconjunctivitis experimentally in human volunteers by using the adenovirus type 8 strain obtained by Jawetz.

The infectious material has been represented by HeLa cell culture fluid of the virus. It has been inoculated in two groups of adult volunteers (of five and three individuals respectively) by instilling the fluid twice a day (every 12 hours) for three days into the conjunctival sac.

The daily examination of the inoculated volunteers, however, did not permit detection of any sign of conjunctival or corneal inflammation in 10 days.

Causes of failure in transmitting the disease could have been: (1) Too low an infectious titer of the inoculated material; (2) inoculation without scarification of the conjunctiva; (3) possible immunity-state of the inoculated individuals; (4) lack of additional factors (herpes virus, trauma, and so forth, according to Jawetz, Thygeson, and coworkers[15]) which could make the infection with adenovirus type 8 effective.

In examining the afore-mentioned hypothesis we think that the presence of neutralizing antibodies cannot be considered as a probable occurrence in every inoculated subject.

Regarding the importance of another factor (herpes virus, for example) it does not

seem to be absolutely necessary in order to provoke the disease, at least experimentally, according to the recent findings of Mitsui.*

We think that the cause of failure in the experimental transmission of epidemic keratoconjunctivitis in our hands can probably be found in the low titer of virus or perhaps in the inoculation technique.

Our apparently negative results in reproducing an experimental disease cannot, therefore, be considered of definite value in assessing the mode of infection with adenovirus type 8 in epidemic keratoconjunctivitis.

Summary

1. Epidemic keratoconjunctivitis was first seen in Italy in 1942, possibly due to the presence of soldiers coming from central Europe where the disease was very prevalent at that time.

2. In the following years epidemic keratoconjunctivitis was encountered in practically every Italian region but rarely with a true epidemic aspect.

3. Our observations cover about 2,500 cases seen in Sassari, Pavia, Parma, and Rome, and show that corneal involvement has a different incidence in different years and areas, and at different ages (being more common in adults), and that aspects different from the typical ones may occur. Antibiotic therapy is considered without effect.

4. A summary of the clinical and epidemiologic patterns of epidemic keratoconjunctivitis in Italy, based upon information gathered in the different parts of the country, is reported.

5. Serologic investigations have been performed on sera of patients with still-active epidemic keratoconjunctivitis or who had had the disease not more than 18 months earlier. For the complement-fixation test an adenovirus type 3 strain was used, while for the neutralization test the adenovirus type 8 strain isolated by Jawetz, Thygeson, and co-workers,[15] was employed.

Complement-fixation tests (group reaction for the adenoviruses) gave 58.7 percent positive results (dilutions of 1:4 or more) in 66 sera. Neutralization tests were positive (at dilutions of 1.32 or more) in 15 out of 19 sera. In further 13 sera of patients with epidemic keratoconjunctivitis from Italy which were sent to Jawetz for examination, the neutralization test was positive (eight times at dilutions higher then 1:10), while it was practically always negative in 24 control sera, also from Italy.

6. The attempts to reproduce the disease in human volunteers did not give definite results, probably owing to the low infectious titer of the infective material.

7. These investigations bring further support to the views which consider the adenovirus type 8 as playing an important role in the etiology of epidemic keratoconjunctivitis.

Addendum

One group of eight volunteers was inoculated with adenovirus type 8 by dropping tissue culture material into one eye. None of them developed any lesions. A second group of six volunteers was inoculated with similar infective material after scarification of the cornea. All six developed follicular conjunc-

---

* One of us (Bietti) had the opportunity during the present experiment to see the results obtained by Mitsui in Japan after inoculation of volunteers with the same adenovirus type 8 strain used by us.

Mitsui inoculated five volunteers and observed in four cases, four to eight days after incubation, an acute conjunctivitis, followed in three cases, eight to nine days later, by a typical keratitis. It should be noted that, in the negative case, in spite of three successive inoculations, the titer of the neutralizing antibodies was 1:20 and 1:40 before the inoculation.

In the positive cases an increase of antibodies was observed (Jawetz and co-workers), including the subject who did show corneal involvement. Bietti examined personally two of the positive patients and could confirm in both cases the typical aspect of the corneal changes of epidemic keratoconjunctivitis, even if the infiltrates were of limited number.

Mitsui was finally able to re-isolate the virus from two of his cases and also to obtain a new strain from a spontaneous case of epidemic keratoconjunctivitis, where the isolation was performed on the second day from the infection.

TABLE 1

SUMMARY OF THE CLINICAL AND EPIDEMIOLOGIC CHARACTERISTICS OF EPIDEMIC KERATOCONJUNCTIVITIS IN ITALY

Locality	Years of Highest Incidence	Seasonal Distribution	Morbidity Differences Between Sexes	Sporadic Cases	Places, Occasions and Modalities of Contagion	Epidemics Among Factory Workers, Metallurgic Works, Shipyards	Mean Duration of the Conjunctival Affection	% Simple Conjunctival Cases	Interval Between Onset of Conjunctivitis and Keratitis	Mean Duration of Corneal Lesions	% of Cases of Unilateral EKC	Particularly Marked Conjunctival Involvement	Pure Nummular Forms not Preceded by Conjunctivitis	Uncommon Corneal Complications	Particularly Marked Inflammation of Anterior Uvea	Concomitance or Succession of EKC. and Extraocular Herpes Febrilis	Effectiveness of Topical Cortisone on EKC	Effectiveness of Drugs
Torino	1947–1948 1951–1952 1955–1956	Winter Time Rapid decrease during spring	Predominant in males	Some cases	Eye dispensaries, hospital wards, ocular surgery	Fiat factory	20 days	10. 7%	7 days (recently even less)	25 days	Minority			Sometimes little epithelial bullas			Effective in association with antibiotics	Chloramphenicol + cortisone
Vercelli	1954	April, May, June			Hospital eye department													
Novara	1955		Predominant in males		Familiar diffusion	Sporadic cases in factory workers	12 days	33%	7 days	25 days	58%	Mucopurulent secretion in 12.8% Parinaudlike cases			In about 19% of cases		Not determinable results	Nil
Genova	1953–1954	From October to March	Exclusively in males	Some cases in September 1953	Eye dispensaries	Severe epidemics in Ansaldo shipyards	7 days	69. 5%	10–15 days	1–3 months	77. 3%	Sometimes abundant secretion					Quite variable results	Terramycin
Como	1954–1955	From December to June	Predominant in males (85%)	Some cases	Familiar diffusion and contagion in eye dept.		15 days	15%	8–15 days	Not easily determinable. Sometimes more than 1 yr.	10%	Mucopurulent and pseudomembranous conjunctivitis		Deep parenchymatous cases	In some cases preceded the onset of punctate sup. ker.	One case (h. simplex labialis)	Not effective	
Pavia	1949 1954 1955 1956	From May to August	None	30–40 yearly	Family, hospital wards or ophthalmic dispensaries	Necchi factory	15–20 days	20%	8–25 days	3–6 months	10%	Pseudomembranous cases					Sometimes unfavorable effect	—
Milano	1946 1955–1956	February and March	None	Some cases yearly Increased 1956	Ophthalmic dispensaries		Few days except in cases of corneal involvement	60–70%	30 days	3–8 months	Some cases						Seems not to be effective	Zinc sulfate 1.5% Sulfonamides per os
Bergamo	1955–1956	Winter time	None	Some cases	Familiar diffusion		8–15 days		Variable, not easily determinable	From some weeks to some months	Nil	Pseudomembranous and pseudotrachomatous cases	Very rare	Superficial ulcerations with rapid evolution			Favorable results	Terramycin or chloramphenicol + cortisone
Brescia			Slightly predominant in males	10–15 yearly	Uncertain causes	Sporadic cases in metallurgic works	12 days	50%	4– 5 days	1–2 months	60–70%	Exceptional abundant secretion	Very rare	Frequent disciform cloudings & dendritic cases. Observed filamentary keratitis	Sometimes slight	In few cases herpes labialis	Favorable results	Ag nitrate Terramycin Lysozyme Cortisone
Verona		None	None							EKC			were				observed	
Padova	1952–1953	January, April May, October	Slightly predominant in women	Few cases yearly	Family and sometimes hospital diffusion		Not determinable	Not determinable	Not determinable	10–30 days	high %			Rare herpetic cases	Cases of iritis			Aureomycin Terramycin
Venezia	1954		none						Further						Data			
Trieste			Predominant in males	10–20 yearly					15–20 days	20–30 days				Relapsing corneal erosions			Effective in absence of corneal ulcerations	Atropine, salicylates bismuth, Vit. B_1 liver extracts
Parma	1951 1954		None	Few cases yearly	Family and school		10–15 days	50%	8–10 days	1–2 months	Majority	Rarely	Sometimes					Ag salts
Bologna	1947 1948 1955 1956	Spring, beginning of summer, autumn	None	10–15 yearly	Family, sometimes hospital wards		10–12 days	15%	7–15 days	30 days	2%	Sometimes muco-purulent secretion	Sometimes	Sometimes transitory marginal or central infiltrates				Terramycin and antibiotics in general
Firenze	1947 1948 1953 1955 1956	Spring and autumn	Predominant in males	50–60 yearly Increased 1956	Family, sometimes hospital wards		10–15 days	60–70% (90–95% during last months)	4– 7 days	20–30 days	30–40% recently increasing	Particularly 1955–1956	Rare cases				Seems to be effective in association with antibiotics	Tetracycline per os Terramycin + cortisone
Livorno	1955	Spring and summer	Predominant in males	4–5 yearly	Ophthalmic dispensaries	Shipyards and factories	21 days	70%	6 days	25 days	25%				Sometimes		Seems to be effective in association with antibiotics	Sulfonamides per os Antibiotics + cortisone
Pesaro				Some cases yearly	School communities (only episode 1950)		15–20 days	60%	4–5 days	20 days	10%			One case of filamentary keratitis			Sometimes worsening of the ocular condition	Aureomycin
Siena	1955	April, May, June	Predominant in males	4–5 yearly	Family and worker communities	Steel plants of Piobino (Magona)	A few days	5–8%	4–10 days	4–10 months						1 doubtful case		Chloramphenicol and antistin-privine
Grosseto	1956	April, May, June	Slightly predominant in women	Some cases yearly since 1947	Family, school, mines		12–20 days	56%		60 days	68%	Pseudomembranous, trachomalike cases. Parinaudlike cases	+ (nonspecific)			Some cases	Sometimes harmful effect	Uncertain effect of antibiotics and lysozyme
Pescara				Few cases yearly			10–15 days		5–10 days	10–15 days	40%							Antibiotics plesio-roentgentherapy
Rieti				About 19–20 yearly	Sometimes familiar diffusion		14 days	Not higher than 5%	7 days	Few weeks to 4–5 months	Rare cases	Some cases with muco-purulent secretion	One case				Effective in final stages	Strepto-penicillin in conjunctival forms
Roma	1942–43 1950 1947 1954 1948 1955 1949 1956	April, May, June	Predominant in women	20–50 yearly	Familiar, sometimes hospital diffusion		8–15 days	82%	8–14 days	15 days to 2–3 months	66. 4%	Pseudomembranous cases. Swimming-bathlike cases	Some cases	Disciform and dendritic-like keratites	23 cases	Rarely	Sometimes harmful effect on kerartitis	Ag salts
Frosinone	1947	September, October, November		5–10 yearly	Sometimes familiar diffusion		15 days		7 days	1–2 months								Zinc sulfate Citrine Atropine
Napoli	1945–1946		None						Further						Data			
Avellino	1950–1951	winter time		Some cases yearly			20–30 days	Few cases		30 days	about 60%	Pseudomembranous and Parinaudlike cases during the epidemics 1950–1951					Unfavorable effect on corneal lesion	Ag nitrate
Bari	1944–1945		None						Further						Data			
Messina	1949 1953	April, May, June	None	5–10 yearly	Family, sometimes hospital wards		14–21 days (longer in keratitis cases)	10–20%	3–5 days (–)	3–4 weeks	20–30%	Rare cases with secretion and hypertrophic cases		Frequent superficial & more rarely deep herpetic cases		In some cases herpes ascertained	No experience	Antibiotics Lysozyme Urotropine
Ligata		Spring and autumn	Slightly prevailing in women	Some cases			13 days	80%	4 days	6 days	65%							Lemon juice Sulfonamides topically
Agrigento		Spring and autumn	Slightly predominant in males	6–7 yearly			6–7 days and even more		4– 7 days	some weeks	25%	Rarely trachomalike forms	Sometimes, rarely	Some disciform				B-complex Antibiotics Cyclotropine
Sassari	1943 1944	From September to December	Predominant in males	On average 20 yearly	Family, sometimes hospital wards		15–20 days	60%	4–10 days	30–40 days	20%	Trachomalike cases			Sometimes			Ag salts and Urotropine

tivitis compatible with epidemic keratoconjunctivitis. Three of them showed superficial keratitis and of these two also developed persisting subepithelial round opacities typical of epidemic keratoconjunctivitis. Among contacts of these volunteers seven additional persons developed spontaneous conjunctivitis of the epidemic keratoconjunctivitis type and two of them also developed typical corneal lesions. Details of these experimental inoculations with adenovirus type 8 will be published elsewhere.

References

1. Acocella, M., and Miani, P.: Osservazioni cliniche e microbiologiche su due episodi di congiuntivite epidemica. Gior. Mal. Infett. & Parassit., **7**:1, 1955.
2. Angelone, L.: La cheratocongiuntivite epidemica in provincia di Frosinone. Ann. ottal. & clin. ocul., **78**:215, 1952.
3. Attanasio, V.: Cheratocongiuntivitie epidemica. Boll. ocul., **25**:481, 1946.
4. Balducci, D., Zaimen, E., and Tyrell, D. A. I.: Laboratory studies of APC and Influenza C viruses. Brit. J. Exper. Path., **37**:205, 1956.
5. Brognoli, C.: Gli antibiotici nella terapia della cheratocongiuntivite epidemica. Rassegna ital. ottal., **21**:121, 1952.
6. Capalbi, S.: Sulla terapia della cheratocongiuntivite epidemica. Arch. ottal., **53**:396, 1949.
7. Casa, G.: Sulla cheratocongiuntivite epidemica. Atti 36 Cong. Soc. oftal. ital., 1947, p. 173.
8. Cavara, V., and Bietti, G. B.: Le manifestazioni oculari delle malattie da virus e da rickettsie, Bologna, Cappelli, 1952, p. 382.
9. Ciotola, G.: Osservazioni cliniche e considerazioni sulla cosi detta cherato-congiuntivite epidemica. Atti 36 Cong. Soc. oftal. ital., 1943, p. 51.
10. Colombo, G. L.: (a) Casi di cheratocongiuntivite nummulare epidemica. Boll. ocul., **35**:297, 1946; (b) Rickettsoide nella cheractocongiuntivite nummulare epidemica. Boll. ocul., **27**:398, 1948; (c) L'importanzadiagnostica della citologia essudativa congiuntivale. Atti 38 Cong. Soc. oftal. ital., 1949, p. 401.
11. Favaloro, G.: Congiuntiviti chemotiche acute non purulente vero-similmente della congiuntivite acuta di Beal o della cherato-congiuntivite epidemica degli autori americani. Communicazione al 3 Congr. Soc. oftal., **29**:6, 1951.
12. Fracissi, G.: La penicillina in terapia oculari. Atti 36 Congr. Soc. oftal. ital., 1947, p. 322.
13. Fulton, F., and Dumbell, K. R.: Quoted by Balducci: J. Gen. Microbiol., **3**:97, 1949.
14. Grignolo, A.: Gli antibiotici in oftalmologia. Torino, Minerva Medica, 1948, p. 131.
15. Jawetz, E., Kimura, S. J., Hanna, L., Coleman, V. R., Thygeson, P., and Nicholas, A.: Studies on the etiology of Epidemic Kerato-conjunctivitis. Am. J. Ophth., **40**:200 (Nov. Pt. II), 1955.
16. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., and Kimura, S. J.: Antibodies to APC virus type 8 in epidemic kerato-conjunctivitis. Proc. Soc. Exper. Biol. & Med., **92**:91, 1956.
17. Lepri, G.: Osservazioni eseguite mediante il microscopio a contrasto di fase sugli elementi cellulari dell'epitelio congiuntivale e corneale in casi di cherato-congiuntivite epidemica. Giorn. ital. oftal., **5**:314, 1952.
18. Panzardi, D., and Pasca, G.: Azione della streptomicina nelle congiuntiviti gonococciche, nelle congiuntiviti da Koch-Weeks, in quelle diplo bacillari e nella cheratocongiuntivite epidemica. Boll. ocul., **26**:581, 1947.
19. Pasca, G.: (a) Su alcuni casi di oftalmia presentate le caratteristiche della cosidetta "cheratocongiuntivite epidemica" recentemente osservati a Sassari. Studi Sassaresi, **21**:115, 1943; (b) Ulteriore contributo allaconoscenza della cosidetta cherato-congiuntivite epidemica (a proposito di oltre 1000 casi osservati a Sassari nel 1943-44). Boll. ocul. **26**:54, 1947.
20. Paternostro, V.: Sull'epidemiologia della cheratocongiuntivite verificatasi nei cantieri navali di Genova-Sestri. Rassegna Med. Indust., **23**:1, 1954.
21. Perria, M.: Sull'epidemiologia della cheratocongiuntivite epidemica. L'Igiene moderna, **48**:117, 1955.
22. Romanzi, C. A.: Isolamento e identificazione del virusdella cheratocongiuntivite epidemica. L'Igiene moderna, **48**:27, 1954.
23. Scardaccione, M.: (a) Considerazioni clinico-statistiche sulla cheratocongiuntivite epidemica nel triennio 1947-49 presso l'ospedale di Grosseto. Gior. ital. oftal., **3**:480, 1950; (b) Caratteristiche della cheratocongiuntivite epidemica (C.C.E.) nella recente epidemia della provincia di Grosseto. Boll. ocul., **35**:521, 1956.
24. Simonelli, M.: Sindrome uveo-meningitica consecutiva a cheratocongiuntivite epidemica. Gior. ital. oftal., **1**:23, 1948.
25. Verderame, F.: Cheratocongiuntivite puntata superficiale epidemica e traumatismo, Rassegna med. infort. & pat. lav., **1**:27, 1948.

THE DIFFERENTIAL DIAGNOSIS OF NUMMULAR KERATITIS (DIMMER) AND EPIDEMIC KERATOCONJUNCTIVITIS*

A. PILLAT, M.D.
Vienna, Austria

Very rarely have two eye diseases been so widely confused as nummular keratitis (Dimmer) and epidemic keratoconjunctivitis. Confusion in the literature has therefore occurred and this has made studies on etiology more difficult. The mistaking of these two diseases is surprising in view of Dimmer's excellent description of his cases in 1905, and in view of Salzmann's presentation of more than 40 masterly illustrations in his detailed work in 1934. These made it possible to see at a glance the characteristics of nummular keratitis, especially its pleomorphism, which distinguish it from epidemic keratoconjunctivitis.

As an excuse for the many misunderstandings in the literature, one can state that nummular keratitis (Dimmer) is limited principally to a relatively narrow territory in the middle of Europe (Pillat, 1940), although the publications of Chen in China and of Woods and Elwyn in the United States indicate that it may also occur in other parts of the world. In any event, most of the authors writing on the subject have referred to the disease without being certain of its clinical appearance (Meisner, Schwitalla, Jancke, and others). It would seem that this error could have been avoided, not only by referring to Salzmann's illustrations of nummular keratitis, but also by following closely the appearance and clinical course of the disease.

All the authors familiar with it, such as Dimmer, Salzmann, Aust, Jesse, Pillat, and others, describe the appearance of the nummular foci on the cornea in association with an almost unirritated and uninflamed conjunctiva. Typically there is no nummular keratoconjunctivitis, only a nummular keratitis; that is, the dramatic conjunctival inflammation which precedes the involvement of the cornea in epidemic keratoconjunctivitis does not occur in nummular keratitis.

I am fully aware that the eyes may occasionally be inflamed in nummular keratitis, and that in epidemic keratoconjunctivitis there may occasionally be only minimal conjunctivitis. I also know that there are occasional severe cases of epidemic keratoconjunctivitis in which the corneal foci may coalesce so as to give a nummular appearance. For such exceptional cases there are special causes which I shall undertake to explain in another paper.

In order to remove the misunderstandings in the literature regarding the morphology of these two corneal diseases, I wish to present pictures and photographs of some of my own cases. I had the opportunity to study two large-scale epidemics of epidemic keratoconjunctivitis in Graz (1942-1944) and in Vienna (1951-1952), and have seen many cases of nummular keratitis in Styria (114 cases) and Vienna during the last nine years.

NUMMULAR KERATITIS

FIGURE 1

Nummular keratitis of several months' duration in the right eye of a man, J. H., aged 31 years (case history 983/40). According to the patient the eye never was inflamed; foreign-body sensation and photophobia during the late fall of 1939 were the only subjective symptoms. The localization of the foci is evident from the figure: most of them are situated in the central part of the cornea but five are peripheral. In spite of the fact that the foci in this case are similar in size and form, one recognizes the different developmental stages and the tendency of the foci to coalesce.

* From the First University Eye Clinic.

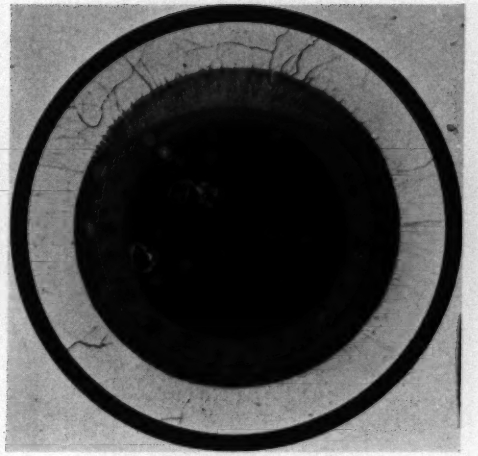

Fig. 1 (Pillat). Nummular keratitis in a man.

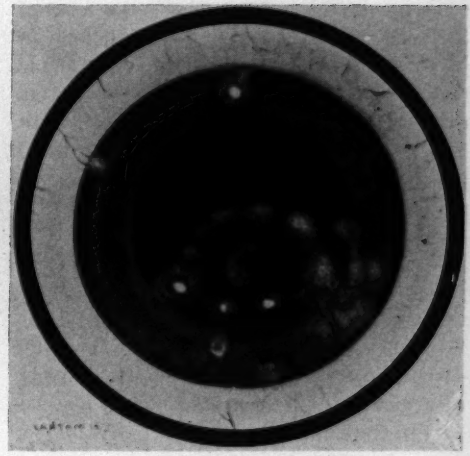

Fig. 2 (Pillat). Nummular keratitis in a woman.

FIGURE 2

Nummular keratitis in a 52-year-old farm woman a few weeks after suffering from foreign-body sensation and epiphora and with a three-week history of diminished vision (case history 262/43).

All corneal foci are diffuse and lie in a common area of opacification which gives the impression of a confluence of the foci. Fresh foci show a dense white center which I have called a "nucleus." In old foci (left side of picture) the center is also dense but only diffusely opaque. In the focus at the 6-o'clock position the resorption of the "nucleus" has led to the formation of an annular opacity.

Central foci are never vascularized. Peripheral foci show superficial new-vessel formation (at the 12- and 7-o'clock positions) and often also deep new-vessel formation (at the 8-o'clock position). Since the nummular foci are nearly always in the anterior quarter of the corneal stroma and only rarely lie deeper, this deep corneal vascularization is also generally in the anterior corneal layers. Figure 2 illustrates a more recent illness than Figure 1 and also shows the different developmental stages of nummular keratitis.

The further course of the disease has been described in the papers already cited:

The corneal foci slowly lose the edematous halo and attain the coinlike form which has given the disease its name, or else coalesce to form larger opaque areas as drawn by Salzmann and as seen in Figures 1 and 2. The "nuclei" of the foci slowly disappear by resorption so that the central part of many foci becomes depressed below the surface of the cornea. These corneal facets, or depressions, cannot be stained by fluorescein because in this stage of the disease the corneal epithelium covering the foci is again normal. The facets can persist for months. The foci in time lose the appearance of dewdrops and form a very fine network of opacities.

During the healing process the peripheral foci disappear first, often as a result of new-vessel formation. In the central foci the cicatrization below Bowman's membrane changes over the years into more or less normal corneal tissue and the opacities may entirely disappear.

Fresh foci of nummular keratitis are often interconnected with fine lines which consist of dewlike points. Such lines can be observed

clearly with the slitlamp above the level of the foci, that is, either in the epithelium or in Bowman's membrane. I have no doubt that they indicate the route of infection, but they are quite different from the dendritic figures of herpes infection of the cornea. I do not agree with Salzmann who concludes on the basis of these connecting lines that nummular keratitis is a herpetic disease, or at least related to herpes corneae. In nummular keratitis the hypesthesia of the cornea so characteristic of herpes is nearly always missing. The linear connection of the corneal foci seems to me to be a general sign of a virus disease of the cornea. It also occurs in epidemic keratoconjunctivitis.

Epidemic keratoconjunctivitis

While I never have observed an eye at the beginning of the subjective signs of nummular keratitis, nor even in the first stages of the corneal lesion itself, it has frequently been possible to observe the involvement of the cornea in epidemic keratoconjunctivitis from its inception to the development of uniform, small corneal foci.

Figures 3 to 6 show the development of epidemic keratoconjunctivitis in a 23-year-old woman.

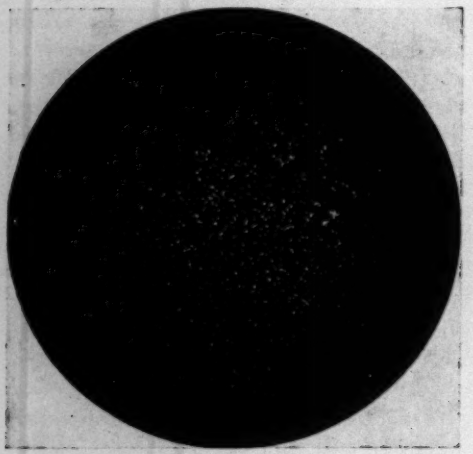

Fig. 3 (Pillat). First stage of epidemic keratoconjunctivitis in a 23-year-old woman.

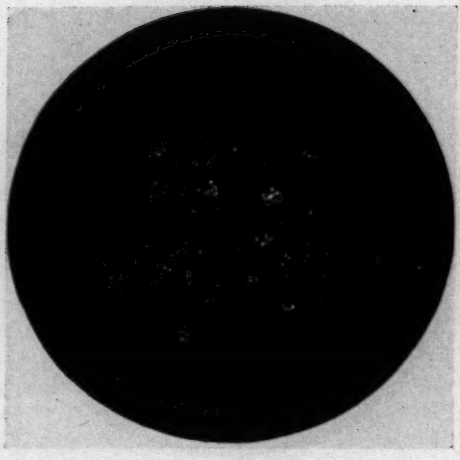

Fig. 4 (Pillat). The second stage in the same patient as in Figure 3.

The first stage of the keratitis (fig. 3) begins while the conjunctivitis, or so-called "Schwellungskatarrh," is at its peak or just diminishing; there is at this time marked involvement of the tarsal conjunctiva and hyperemia of the bulbar conjunctiva. Initially the corneal epithelium is roughened and the reflex is somewhat dull and irregular. When exposed to air, the central corneal epithelium dries out after 10 to 20 seconds. Within the first 24 hours fine epithelial punctate opacities appear.

In the second stage (fig. 4) the corneal epithelium becomes irregularly opacified and the foci stain with fluorescein. These foci are still in the epithelium but behind them the superficial layers of the corneal stroma are already edematous. This epithelial infiltration usually spreads in the next day or two over most of the cornea and presents a picture approximating that of a keratitis punctata superficialis epithelialis (fig. 5) whose foci lie between the punctate epithelial opacities mentioned in the description of Stage 1 and are uniform in shape.

This condition develops into Stage 3 (fig. 6) within 24 to 48 hours. This stage is characterized by small, subepithelial, grayish,

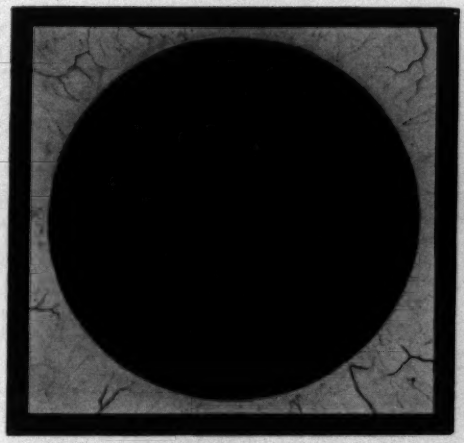

Fig. 5 (Pillat). Beginning of epithelial infiltration.

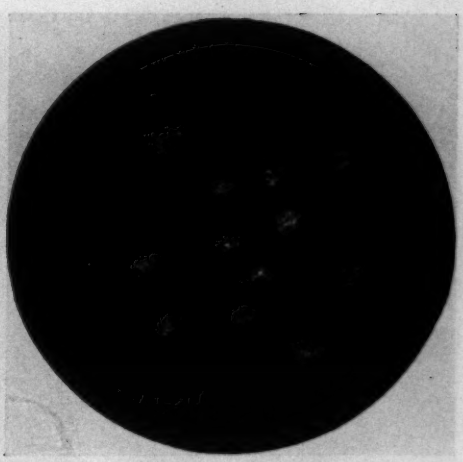

Fig. 6 (Pillat). The third stage.

generally uniform, disclike foci lying under Bowman's membrane. At this stage the epithelial involvement, which provides the basis for regarding epidemic keratoconjunctivitis as a form of superficial punctate keratitis, disappears.

As in nummular keratitis, the small subepithelial foci are in the beginning composed of dewdroplike spots which sometimes develop "nuclei," and which are also in many cases joined together by fine lines which lie more on the surface than the disclike foci themselves. The small discs are at first indistinct but are later sharply circumscribed and show a tendency to coalesce only in exceptional cases. Even when they are close together and numerous (30 to 70 foci), they remain distinct and separated.

In epidemic keratoconjunctivitis minimal vascularization of the cornea can also appear in foci near the limbus. Even then the foci can be seen by slitlamp examination to lie directly under the epithelium in the superficial corneal layers. As the number of disciform corneal foci is almost always smaller than the number of epithelial foci in Stage 2, and since these corneal foci usually show an even distribution in the central part of the cornea, I believe that the neurotropic virus reaches the superficial corneal layers from the epithelium by way of the rami perforantes of the corneal nerves through Bowman's membrane. In epidemic keratoconjunctivitis the slight depression of the corneal surface so characteristic of nummular keratitis can be observed only rarely. The resorption of the corneal foci can be complete in from six to eight weeks but in our experience usually lasts from six months to two years.

To remove all doubts arising from any inaccuracies that may have occurred in the reproduction of the drawings, I submit the following six photographs of the anterior sector of the eye which I made with the Zeiss stereocamera. Three cases of nummular keratitis (figs. 7 to 9) and three cases of epidemic keratoconjunctivitis (figs. 10 to 12) were selected arbitrarily for these photographs which are best viewed in pairs. Pertinent parts of the stereophotos have been enlarged so that the magnification of the cornea is constant. These photographs show clearly the following:

1. That on the average the foci in nummular keratitis are substantially larger than the foci in epidemic keratoconjunctivitis.

2. That corneal foci in nummular keratitis

Fig. 7 Fig. 8 Fig. 9

Figs. 7, 8, and 9 (Pillat). Three cases of nummular keratitis.

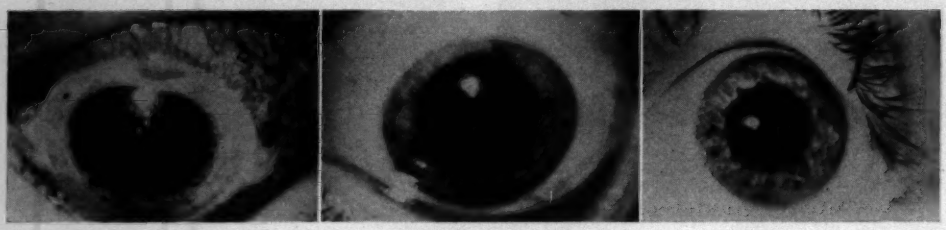

Fig. 10 Fig. 11 Fig. 12

Figs. 10, 11, and 12 (Pillat). Three cases of epidemic keratoconjunctivitis.

are usually more dense, that is, whiter, than the corneal foci in epidemic keratoconjunctivitis.

3. That the lesions of nummular keratitis are less uniform than those of epidemic keratoconjunctivitis.

4. That the nummular foci have more of a tendency toward confluence (figs. 7 to 9), even when they are less crowded together (fig. 8), than the foci of epidemic keratoconjunctivitis (fig. 10).

In conclusion, a few characteristic signs of nummular keratitis and epidemic keratoconjunctivitis are listed for comparison. This list and the photographs establish that we are dealing with two different corneal diseases. That certain details can be common to both diseases, for example, the development of fresh foci from dewdroplike dots, and the intercommunication of single foci through fine lines, confirms my opinion that in both instances we are dealing with a virus infection. The interconnection of individual foci by fine lines does not occur in corneal lesions caused by bacteria or fungi. I believe, therefore, that this feature is peculiar to viral diseases of the cornea (Pillat, 1943).

Summary

The differential diagnosis (table 1) of nummular keratitis and epidemic keratoconjunctivitis is presented by means of short descriptions of the clinical manifestations of the two diseases and by illustrations.

To offset the possibility of subjective mistakes by the illustrator, enlarged photographs of three corneas with nummular keratitis and of three corneas with epidemic keratoconjunctivitis are submitted. These enable the observer to see clearly the morphologic differences between the two diseases.

A comparison of the principal symptoms of both diseases, and an analysis of the anamnestic and clinical data, should help in the differential diagnosis.

Nummular keratitis and epidemic keratoconjunctivitis are two unrelated diseases of the cornea which are probably both caused by viruses. The causal viruses are unrelated to each other or to herpes virus.

First Eye Clinic.

TABLE 1

DIFFERENTIAL DIAGNOSIS OF NUMMULAR KERATITIS AND EPIDEMIC KERATOCONJUNCTIVITIS

Signs	Nummular Keratitis	Epidemic Keratoconjunctivitis
Population	Almost exclusively farmers	Principally city dwellers
Causes ascribed by the patient	Result of minor lesions sustained during farmer's activity (threshing, hay-making, etc.)	Following small ophthalmological interventions and operations
Form of occurrence	Sporadically; some years more abundant	Epidemically; also isolated cases
Seasonal appearance	Spring and autumn	?
Geographic occurrence	Austria: eastern margin of Alps as far as white Carpathians; Indonesia, China, United States	Everywhere in the northern hemisphere
Systemic involvement	None	Usually slight influenza-like illness
Lymphadenopathy	Not observed	An almost constant feature
Sex	No recognizable predilection for either sex	
Age	All ages	Middle age
Infection of one or both eyes	Usually one eye only	Usually both eyes
Conjunctival reaction	None, but nothing is known about onset	Severe conjunctivitis 6 to 10 days before keratitis
Onset of corneal lesion	Slow, creeping	Sudden
Size of corneal foci	0.5 mm. to 2.0 mm.	Usually smaller than 0.5 mm.
Form of corneal foci	Roundish but irregular owing to coalescence	Round
Number of corneal foci	1–10, occasionally 20	10–60
Facet formation	The rule	Rare
Thickness of corneal foci	1/5 to 1/3 of the corneal thickness	1/8 to 1/5 of the corneal thickness
General aspect of the cornea	Pleomorphic lesions	Uniform lesions
Involvement of the deeper layers of the eye	None	Rare in severe cases
Vision	Diminished when large corneal foci develop	Initially diminished, later normal
Late course in corneal foci	Disappear in from 2 to 8 years	Disappear in from 8 weeks to 2 years

KERATITIS SUPERFICIALIS TROPICA

A DISEASE OF SOUTHEAST ASIA CLOSELY RESEMBLING EPIDEMIC KERATOCONJUNCTIVITIS

THE COMMITTEE* ON KERATITIS SUPERFICIALIS TROPICA
Colombo, Ceylon

1. Keratitis superficialis tropica is the name given locally to a common disease of southeast Asia which is characterized by a nonpurulent conjunctivitis followed by a keratitis with corneal opacities. In this respect it closely resembles epidemic keratoconjunctivitis, yet at the present moment there are enough differences to justify a difference in nomenclature. The problem to be decided is whether keratitis superficialis tropica as seen in Ceylon is an infectious disease and if so whether it is related to epidemic keratoconjunctivitis. In February, 1956, a study was commenced in Colombo with the objective of determining the possible relationship of keratitis superficialis tropica to epidemic keratoconjunctivitis and this paper incorporates the findings of the past four months.

2. Classical epidemic keratoconjunctivitis is common in the tropical parts of Asia, and very large epidemics have been described. In published reports, there seem to be no major variations between the disease in Asia and America. Keratitis superficialis tropica is distinctive in that, superficially at least, there is no evidence of person to person transmission, and many physicians regard it as noninfectious.

3. The disease is very common in Ceylon. At the Victoria Memorial Eye Hospital, Colombo, which has 200 beds and an average of over 400 out-patients a day, there were, in 1954, 2,240 cases of keratitis superficialis tropica seen as out-patients and 156 treated as in-patients. In 1955 the out-patient figures had increased to 3,479. Approximately 100,000 treatments a year are given in the dispensaries scattered through the island. In a country with a population of less than eight million, this represents a substantial attack rate, and if the disease is present in the same proportions in other countries in southeast Asia, then it is a major medical and public health problem in this area.

4. A comparison of keratitis superficialis tropica as seen in Ceylon in 1956 with classical epidemic keratoconjunctivitis reveals the following differences:

1. There has been no history of contact of any of the cases studied with other cases, although numerous patients have been closely questioned on this point. Three epidemics of reported keratitis superficialis tropica have been investigated but all proved to be caused by pus-forming organisms.

2. All cases of keratitis superficialis tropica except one investigated in the past four months have had only one eye affected, whereas in epidemic keratoconjunctivitis commonly both eyes are involved.

3. In no instance has the preauricular gland been enlarged.

4. Sometimes the opacities in the cornea are very much larger in keratitis superficialis tropica than in epidemic keratoconjunctivitis, so that occasionally up to 50 percent of the cornea may be affected.

Apart from these points, the two diseases are very similar, both having a nonpurulent conjunctivitis followed by a keratitis with corneal opacities and no staining with fluorescein. In most cases seen individually, the diagnosis could be either keratitis superficialis tropica or epidemic keratoconjunctivitis. Specimens are being collected for testing against the group of adenoviruses in the hope of discovering whether there are two separate diseases in southeast Asia, or merely variations of one.

* T. R. Jansen, chairman, Victoria Memorial Eye Hospital, Colombo, Ceylon; A. Viswalingam, 69/1 Ward Place, Colombo, Ceylon; P. Sivasubramaniam, A. T. Navaratnam, O. L. F. Senaratne, and K. J. L. de S. Deva Aditiya, Victoria Memorial Eye Hospital, Colombo, Ceylon; P. Silva; and T. A. Cockburn, c/o WHO, Box 1505, Colombo, Ceylon.

ETIOLOGY OF EPIDEMIC KERATOCONJUNCTIVITIS BASED ON 1942 STUDIES*

MURRAY SANDERS, M.D.
South Miami, Florida

The present communication is limited to experiences involving studies of possible etiologic agents in the New York State epidemic keratoconjunctivitis (EKC) outbreaks in 1942. Dr. F. S. Cheever[1] has kindly sent me his manuscript in advance of this meeting, and, with one exception,† it is clear that a careful history of epidemic keratoconjunctivitis events subsequent to 1942 has been recorded. In this report, consideration is also briefly given to several clinical facets of the disease, but only in respect to potential effects on pathogenesis.

It would be unrealistic not to recognize there have been points of confusion which arose in the field of virology subsequent to the isolation of a filterable agent purported to be the cause of the New York cases of epidemic keratoconjunctivitis, and the current symposium is a good opportunity to correlate, before a scientific group, the 1942 experiences.

Primarily, consideration must be given to objectivity so that errors, if there were any, are not repeated. On the other hand, correlative data may reveal interesting and useful relationship within the field of virology.

The clinical aspects of epidemic keratoconjunctivitis have been adequately discussed in the present symposium. Mention of clinical observations is here made incidental to the 1942 outbreak in New York State only where they may have some bearing on immunologic and etiologic aspects.

Aside from the characteristic ocular involvement, the following points were noted in New York in 1942:

1. Lymphadenopathy was common and pronounced, and of several weeks' duration. One or more, or all, groups of the following glands were involved—preauricular, cervical, and submental.

2. Headaches were frequently associated with the ocular disease, pain varying in intensity and sometimes beginning within 36 hours after the onset of the conjunctivitis. It was noted the headaches were not relieved by analgesics.

3. Rhinitis and sore throats were occasionally noted.

4. Two patients from Schenectady[2] showed possible central nervous system involvement, marked by persistent and extreme drowsiness. It was not unusual to find malaise and fever in conjunction with the ocular disease.

It is pertinent to present considerations that there may have been an increase in virulence of the agent responsible for the New York disease in 1942.[3] In the outbreak of epidemic keratoconjunctivitis, first seen on the west coast, the patients were usually incapacitated for one to two weeks, and corneal opacities of a transient character appeared in 40 to 75 percent of the cases. After passing through human hosts in the midwest, and apparently finding an unusually fertile soil in the crowded factories of the east, the disease appeared to be more severe in the New York area than in California, judged by the more distressing subjective symptoms and the extended nature of the

* From the Department of Microbiology, University of Miami.

† Cheever has properly emphasized the lack of Patient E. L.'s serum to neutralize the virus received from New York. E. L. was the source of one of the early isolations. The serum test may or may not be valid. The conditions under which serum was obtained should be noted. When E. L. was finally located 10 years after his epidemic keratoconjunctivitis infection, he was practicing ophthalmology in Massachusetts. He was entirely co-operative, but apparently had little experience in collecting serum. A whole-blood sample received in Miami from Massachusetts, after several days en route had obviously been subjected to considerable heat. This should be considered a poor sample for neutralization test. Nevertheless it was sent to Cheever without comment.

acute stages, which lasted frequently for three or more weeks. Furthermore, the opacities were observed in as high as 85 to 90 percent of the cases in the east, and there was a tendency for these corneal subepithelial spots to persist for many months.

In March, 1942, a virus was presumably isolated from the eyes of two patients suffering with epidemic keratoconjunctivitis at the College of Physicians and Surgeons, Columbia University, New York City. The diagnosis was made in each instance by Dr. Phillips Thygeson, co-director of the Institute of Ophthalmology, Presbyterian Hospital, New York. Dr. Thygeson also made available scrapings from the conjunctival surfaces of these patients for staining, and for inoculation of material into tissue cultures and into mice. The details of the isolation have been published,[4,5] and it is known that a filterable agent was isolated after primary incubation of the conjunctival scrapings in tissue cultures, and by subculturing embryonic mouse brain in tissue cultures. By intracerebral injection of scrapings or of emulsified tissue culture material containing scrapings into mice, transient symptoms were noted. On a number of occasions conjunctival scrapings from normal individuals, or from persons suffering from nonbacterial infections of the eye other than epidemic keratoconjunctivitis, were submitted to the tissue culture-mouse intracerebral technique. The course of events in such instances was quite different from that noted in epidemic keratoconjunctivitis experiments.

That the agent under discussion was not easily isolated is apparent from one series of experiments[6] where only two of 19 isolation efforts resulted in demonstrating a mouse to mouse transmissible virus.

In the first virus isolation, the brain tissue from sick mice contained a demonstrable filterable agent after passage through embryonic mouse brain-serum ultrafiltrate cultures.

The virus which was established in mice had an intracerebral LD_{50} between 10^{-5} to 10^{-6} and the incubation period in mice varied from three to seven days, depending on the dilution injected. In tissue culture, the agent was maintained at 37°C. for three days before subculture, but the best results were obtained with room temperature cultures after seven days. The virus was pathogenic for unweaned Swiss mice injected by intranasal, intraperitoneal, and intracerebral routes. For adult mice, however, only intranasal and intracerebral routes could be used for transmission of the agent, and in the case of rabbits only the intracerebral route was effective. The virus was not pathogenic for guinea pigs and albino rats (strain unknown).

It is to be noted that the activity of the virus in the tissue cultures was of a low order of magnitude, varying from 10^{-2} to 10^{-3} in the majority of cultures, and only when the tissue was emulsified.

The specific nature of the virus which was isolated in New York appeared to be established by neutralization with known convalescent serum, and the failure of neutralization to occur with serum specimens taken in the acute stages of the disease. This will be discussed later.

On the basis of filtration experiments the agent appeared to be a small virus, since it passed with ease through a double Seitz filter and through collodion membranes with known average A.P.D.s. The graded collodion membranes which were used were obtained from Elford in England, and were checked for A.P.D. size before being used. Through these membranes the virus failed to pass when the A.P.D. was 25 to 30 mμ, but it was not held back when the A.P.D.s were 50 to 75 mμ.

Only a single human volunteer was tested with this laboratory agent. In view of subsequent failures (with three possible exceptions) to obtain similar strains of virus from epidemic keratoconjunctivitis patients, the human case is considered in some detail. Furthermore, although the point has not been made in 12 years, since Dr. Cheever has

mentioned my role in the laboratory infection, it can be stated that I was the human volunteer.

On July 3, 1942, about 3,000,000 intracerebral doses of the mouse virus were inserted on the conjunctiva of the right eye, and with the eye closed the upper lid was rubbed for about one minute. A patch was kept over the eye for 30 minutes and two more drops of the infectious material were added. The source of the infection was a mixture of the 15th tissue culture generation containing a suspension of mouse brain from the first isolation. At that point Dr. Thygeson was informed of the experiment, and he observed the eye of the volunteer frequently.

After a four-day period no significant symptoms were noted, although a mild conjunctivitis with a few follicles was present. The cultures on secretion were negative and smears showed a few mononuclear cells without bacteria. There was no edema of the conjunctiva, nor was the preauricular gland on the infected side enlarged. After the four-day asymptomatic period, washings from the right eye were put into the left eye and additional infected mouse brain emulsion was rubbed into the lower conjunctiva of the right eye with a swab. One week after the first instillation of infectious material, and three days after the second, a diagnosis of epidemic keratoconjunctivitis was made at the Ophthalmological Institute, New York (fig. 1).

In view of the mild nature of the disease at that time, the situation was highly satisfactory to all concerned. However, a week later, that is, two weeks after the beginning of the experiment, there was a painful sensation of a foreign body in the eye and what appeared to be an acute exacerbation ensued. Lacrimation was copious, and both the preauricular and submental glands on the right side were tender. The submental node was about the size of an almond. Nineteen to 26 days after the beginning of the experiment there was a general decrease in objective signs, with a diminution of mucous membrane hypertrophy. Moderate infiltration and hypertrophy persisted, and several corneal opacities were noted in the infected eye. These opacities were not stained by fluorescein and were considered characteristic of epidemic keratoconjunctivitis. The opacities did not disappear for more than one year.

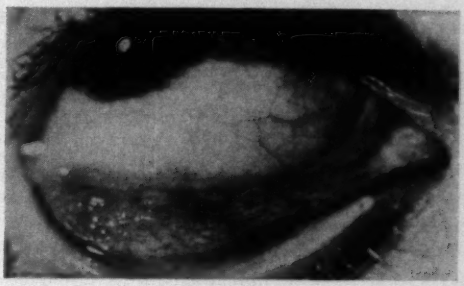

Fig. 1 (Sanders). Right eye of human volunteer, one week after infection with the virus isolate of New York, 1942. Note follicles and exudate on edematous and hyperemic conjunctiva. (Printed with the permission of the editors of the *Journal of Experimental Medicine* from reference #5.)

One of the most inexplicable aspects of the 1942 experience with epidemic keratoconjunctivitis at Columbia, in view of difficulties which arose later, was the apparently straightforward, consistent, and specific neutralization of the New York virus by convalescent serums from known epidemic keratoconjunctivitis patients. By agreement between Mrs. Alexander and me serum tests were run by number as much as possible.

At the present writing, July, 1956, the total number of serums which were tested "blindly" cannot be given because of the lack of records dating back to 1942. However, it is a matter of published record[7] that 118 serums were tested, by number, for neutralizing antibodies against the New York virus, and the results strongly suggested that the New York virus was related to the disease recognized as epidemic keratoconjunctivitis. It is to be emphasized that the serums tested in this study, and tabulated below (table 1), were derived from both the New York and the Schenectady areas. A number of the pa-

TABLE 1*

EXTENT OF NEUTRALIZATION TO THE VIRUS OF EPIDEMIC KERATOCONJUNCTIVITIS SHOWN BY SELECTED SERA

Titer of Serum	No. of Neutralizing Doses	Cases			Contacts		Control No. Known E.K. or Contact	Total
		Early 1st Week	Convalescent 6–10 Weeks	Late 4–5 Months	Intimate (5 mo. after Onset of Case)	Remote		
10^{-6}	0	30	..	4	7	4	28	73
10^{-5}	10	1	..	2	4	4	1	12
10^{-4}	100	..	..	2	3	..	..	5
10^{-3}	1,000	..	14	2	3	..	..	19
10^{-2}	10,000	..	7	..	..	..	..	7
10^{-1}	100,000	..	2	..	..	..	..	2
Total		31	23	10	17	8	29	118

* Korns, R. F., Sanders, M., and Alexander, R. C.: Epidemic keratoconjunctivitis: Correlation of epidemiologic data and results of serum virus neutralization tests. Am. J. Pub. Health, **34**: 567–571 (June) 1944. (Reprinted with the permission of the editors.)

tients in Schenectady were vaccinated with the New York virus as part of a study carried out during the epidemic. However, there is little question that if any of these patients were included in the serum tests, the number must have been small.* Furthermore, the serum-virus studies in question could at best have been influenced by vaccination only to a minor degree, since individuals in New York City who contributed to these tests were not vaccinated. An attempt was made to learn specifically the number of the patients from Schenectady who might have received New York virus, and who would have been in the 23 positive neutralization tests recorded in Table 1 since the evidence for neutralization was strong in this group and varied in individual serums from two to four logs of virus.

The technique for neutralization has been described[5] and need not be repeated here. What is more important in attempting to evaluate the 1942 puzzle is that in addition to the 118 serums mentioned above, the following serums from epidemic keratoconjunctivitis patients were tested against the New York virus and yielded consistent immunologic data:

A. Three serums from California sent by Dr. Hogan and taken one to six months after the acute disease.[5]

B. Seven paired serums from New York patients, that is, acute and convalescent phase specimens.[5]

C. Nineteen convalescent serums from a localized outbreak in New York City.[6]

It is to be noted that of the 19 convalescent serums last mentioned, six were paired and showed a rise in antibody titer against the New York virus.

In addition to specific tests against epidemic keratoconjunctivitis serums, numerous tests were carried out against serums from unrelated keratitides and various types of conjunctivitides. In no instance was there an indication of in vitro immunologic reaction similar to that seen in epidemic keratoconjunctivitis. Similarly, tests against lymphocytic choriomeningitis and Theiler's mouse virus made it clear we were not dealing with the well-known latent viruses.

DISCUSSION

In summary, it is easily understood, on the basis of the published record, why the workers who isolated the presumptive causal virus for epidemic keratoconjunctivitis in New York in 1942 were of the opinion that the problem was simple, and that the pro-

* The number and type of serums tested from Schenectady and New York City have been clarified by Dr. Korns and Dr. Braley.

cedures employed were straight-forward. It was noted at various times that the virus in question was not easily isolated, but certainly the difficulties which arose later were not anticipated. It is a matter of record that any qualified worker who requested a sample of the New York virus or serums received co-operative action as soon as possible. Perhaps the most confusing aspect of the entire situation was the herpes strain which Dr. Edward Lennette apparently received from my laboratory. That we were not dealing with herpes simplex seemed definite, simply on the basis of filtration studies and the large number of negative neutralization tests which were obtained against epidemic keratoconjunctivitis acute phase serums or normal serums.

While it has been stated that there was no confirmation, or at best questionable confirmation, of the work in New York, there are several points that should be made in reference to this. It is my opinion that confirmation was obtained in the laboratories at the Johns Hopkins Hospital by Maumenee who reported on the herpeslike character of epidemic keratoconjunctivitis.[8] If Maumenee's work is carefully examined, the so-called "Case 1" or isolate No. 1 is interesting. This virus was filtered with ease through double Seitz pads and . . . "animals completely immune to herpes are killed by intracerebral inoculation of the E.K. virus." This statement regarding E.K. virus made by Maumenee refers both to a virus sample sent by me, as well as to the virus isolated from Patient 1. There is little question that the other isolates of Maumenee were herpes, but it appears quite likely that the first strain was closely related, or identical, to the New York agent.

Also, in relation to Maumenee's work, the statements of Rake[12] about herpetic infections are pertinent. Rake has emphasized that herpes is an ubiquitous agent with significant neutralizing antibody levels occurring in 90 percent of individuals over 15. Furthermore, he notes that herpetic isolates must be carefully evaluated as causative agents of disease. With these facts in mind, the course of Maumenee's No. 1 isolation should be re-evaluated. The virus derived from the first patient was the only one of six strains in which inclusions were not correlated with infection and keratitis in rabbits and could not be induced with early passage material. Clinically, the first patient was the only one of six in whom subepithelial, nonstaining infiltrates were noted.

Since Maumenee's strains, Nos. 2, 3, 4, 5, 6, were almost certainly herpes, it is important that strain No. 1 retained individual characteristics. It is most unfortunate that this strain was not available for later study. This is a part of the Columbia puzzle too, since it is to be noted that strain No. 1 was sent to New York and the responsibility is therefore bilateral.

Other facets of confirmatory attempts have been well handled by Dr. Cheever, and need not be repeated here. Unfortunately, although it seemed that every reasonable precaution had been taken to insure maintenance of the virus, my absence for four and one-half years (1943-1947) from the laboratory at the College of Physicians and Surgeons, made it impossible to check back and to clarify points of confusion. What happened to the potent rabbit hyperimmune serum pool, and to various specimens of virus cannot be explained.

Calkins and Bond[13] investigated the New York virus in eggs and found it was neutralized by both the New York rabbit hyperimmune and New York human convalescent serum. Again, attention is directed to the inexplicable course of events in connection with the New York virus. When the reports of the egg work and the neutralization data were received, the data appeared to be related to a stable and reliable agent. Dr. Cheever has reported, however, that one source of his St. Louis encephalitis-related strain came from Calkins and Bond *via* Braley. It is this observation which is the most cogent one, in my opinion, that per-

haps, after all the 1942 virus was indeed antigenically related to St. Louis encephalitis.

The fact must be recognized that subsequent to 1942 an agent similar to that isolated in New York, was not unequivocally obtained. The original workers have no doubt that the virus with which the hundreds of neutralization tests were carried out, and which produced in the human volunteer the characteristic course of epidemic keratoconjunctivitis, was the causative agent for the outbreaks of the disease in 1942, or was at least in some fashion related to the epidemic keratoconjunctivitis of 1942. There apparently are epidemic keratoconjunctivitis viruses which fall into the adenovirus group,[9] but a mouse pathogen, as described in 1942, should still be sought. It is a coincidence that in the early part of July, 1956, a communication was received from a capable virus worker who wrote he believed he had isolated an agent similar to the one described by Mrs. Alexander and me in 1942, but he stated he wished to study the agent for a longer period of time, and he will undoubtedly report when the data are unequivocal. It appears that this agent is not adenovirus type 8. Its titer in mice and its host range, as well as its general behavior, encourage one to believe there still may be clarification of the present puzzle regarding the original virus.

Whether our agent was related to St. Louis encephalitis is not clear. No tests were carried out in 1942 to determine this relationship, for the simple reason it did not occur to us to do so. As noted by Cheever, to the best of our knowledge we did not handle St. Louis encephalitis prior to 1950, either in the laboratory or in the field.

Finally, it is desired to comment on the techniques used for isolation of the 1942 virus under discussion. In this symposium, Jawetz and his colleagues[10] have set up criteria "which must be fulfilled before a claim for the identification of *the* etiologic agent of a viral disease . . . can be taken seriously."

The techniques for isolating the 1942 virus followed a classical pattern which forms the basis of virology. Indeed, more than 40 agents have been isolated[11] from human and animal virus diseases by combining the use of tissue culture, fertilized egg techniques, and inoculation of experimental animals. On the whole they have been successful to a gratifying degree and it is safe to say they will not be discarded for some time. If the interpretation of the criteria of Jawetz et al.[10] is correct, there is an inference that transmission of isolate materials "in passage" is hazardous and inferior, whereas the newer, cytopathic techniques represent a more, or most desirable approach to isolation procedures. Certainly recent trends in virology emphasize CPE and the present symposium is a good example of reliance on cytopathic changes.

In this report it is desired to express what is obviously a minority opinion. It is true that occasionally the human factor is responsible for loss of virus strain or confusion regarding its classification. But surely this can happen with any technique. One need only refer to any standard virus text to find such examples, for example, Australian X. However, granting that cytopathic findings are useful, it is important to maintain an open mind about all techniques used for isolation of viruses. The possibility should at least be considered that the role of the experimental animal has been dangerously minimized; and contrariwise the role of cytopathic agents may be dangerously exaggerated. The latter point should be viewed in relation to causative agents of disease, where multiple viruses may be carried in tissue culture systems, particularly noncytopathic change producers. For this reason the plaque technique of Dulbecco[14] and his associates should be used wherever possible so that single genetic units can be tested for their capacity to produce disease as well as uniformity of CPE.

University of Miami.

REFERENCES

1. Cheever, F. S.: The relationship of the epidemic keratoconjunctivitis (EKC) virus of Sanders to St. Louis encephalitis (SLE) virus. Symposium on viral keratoconjunctivitis, San Francisco, Sept. 7-8, 1956.
2. Simmons, J. S., Dunnington, J. H., Sanders, M., Braley, A., Perkins, J., Paul, J. R., and Lanza, A. J.: Symposium on epidemic keratoconjunctivitis, New York, Dec. 4, 1942.
3. Sanders, M.: Epidemic keratoconjunctivitis. Scient. Month., **57**:469-472, 1943.
4. ———.: Epidemic keratoconjunctivitis (shipyard conjunctivitis): Isolation of a virus. Arch. Ophth., **28**:581-586, 1942.
5. Sanders, M., and Alexander, R. C.: Epidemic keratoconjunctivitis: 1. Isolation and identification of a filterable virus. J. Exper. Med., **77**:71-96, 1943.
6. Sanders, M., Gulliver, F. D., Forcheimer, L. L., and Alexander, R. C.: Epidemic keratoconjunctivitis: Clinical and experimental study of an outbreak in New York City: Further observations on the specific relationship between a virus and a disease. J.A.M.A., **121**:250-255 (Jan.) 1943.
7. Korns, R. F., Sanders, M., and Alexander, R. C.: Epidemic keratoconjunctivitis: Correlation of epidemiologic data and results of serum-virus neutralization tests. Am. J. Pub. Health, **34**:567-571, 1944.
8. Maumenee, A. E., Hayes, G. S., and Hartman, T. L.: Isolation and identification of the causative agent in epidemic keratoconjunctivitis (superficial punctate keratitis) and herpetic keratoconjunctivitis. Am. J. Ophth., **28**:823-839 (Aug.) 1945.
9. Jawetz, E., Kimura, S. J., Hanna, L., Coleman, B. A., Thygeson, P., and Nicholas, B. S.: Studies on the epidemiology of epidemic keratoconjunctivitis. Am. J. Ophth., **40**:200-209 (Nov., Pt. II) 1955.
10. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., and Kimura, S. J.: The etiology of epidemic keratoconjunctivitis. Symposium on viral keratoconjunctivitis, San Francisco, Sept. 7-8, 1956.
11. Sanders, M., Kiem, I., and Lagunoff, D.: Cultivation of viruses. Arch. Path., **56**:148, 1953.
12. Rake, G. W.: The etiological role of the virus of herpes in ophthalmic disease. Symposium on viral keratoconjunctivitis, San Francisco, Sept. 7-8, 1956.
13. Calkins, H. E., and Bond, G. C.: Adaption of virus of epidemic keratoconjunctivitis to development in extra-embryonic fluids of chick embryo. Proc. Soc. Exper. Biol. & Med., **56**:46, 1944.
14. Dulbecco, R., and Vogt, M.: Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exper. Med., **99**:167-182 (Feb.) 1954.

THE RELATIONSHIP OF THE EPIDEMIC KERATOCONJUNCTIVITIS VIRUS OF SANDERS TO THE ST. LOUIS ENCEPHALITIS VIRUS*

F. S. CHEEVER, M.D.
Pittsburgh, Pennsylvania

The purpose of this paper is to review the evidence bearing on the relationship of the virus of epidemic keratoconjunctivitis isolated by Sanders[1] in 1942 in New York City, to St. Louis encephalitis virus. Inasmuch as Dr. Sanders has described the isolation of this agent in a previous paper on this program and Dr. Korns is to review the epidemiologic evidence bearing on the possibility that this agent was indeed the cause of the large scale outbreaks seen in 1942 and 1943, no further mention of these phases will be made here. The first part of this paper will be devoted to a chronologic account of the events leading to the discovery of the antigenic relationship of the two agents. The second part will summarize the more important similarities and differences between the two agents. The third part includes a brief account of subsequent efforts to link these viral agents (epidemic keratoconjunctivitis virus of Sanders and St. Louis encephalitis virus) to outbreaks of clinical epidemic keratoconjunctivitis.

In 1947, Dr. Heyl, Dr. Allen, and myself[2]

* From the Departments of Epidemiology and Microbiology, University of Pittsburgh, Graduate School of Public Health.

were engaged in studies aimed at determining the neutralizing antibody content of various pools of gamma globulin against the viruses of herpes simplex and lymphocytic choriomeningitis. Inasmuch as Maumenee, Hayes, and Hartman[3] had recently reported that an antigenic relationship existed between the viruses of epidemic keratoconjunctivitis and herpes simplex it was thought desirable to determine if the various pools of human gamma globulin possessed neutralizing antibody against this latter virus as well.

Attempts to obtain a sample of epidemic keratoconjunctivitis virus from Dr. Sanders failed; he reported that all his stocks of virus and of specific antisera had been inadvertently discarded while he was on military service during the Second World War. We were finally successful in obtaining a strain of the virus from Dr. Sanders' associate, Dr. Braley. According to the latter,[4] this strain had been originally isolated by Dr. Sanders and carried through an undetermined number of mouse and egg passages. During the Second World War it was sent to the Upjohn Company in Kalamazoo, Michigan, where it was carried through another series of mouse and egg passages.[5] At the end of the war it was returned to Dr. Braley who confirmed its specific identification by neutralization tests and filtration studies.[4]

This strain when received through the kindness of Dr. Braley was labeled EK-TC 17. We employed Dr. Braley's designation (EK-TC 17) throughout our studies; subsequently Dr. Ruchman has referred to it as the "Cheever" strain. Our studies with gamma globulin showed that no neutralizing effect could be demonstrated against the lymphocytic choriomeningitis and epidemic keratoconjunctivitis viruses; on the other hand a definite though not marked neutralizing effect could be demonstrated against the virus of herpes simplex.[2] The results of these studies (which were carried out in vitro) failed to demonstrate evidence of an antigenic relationship between herpes simplex and epidemic keratoconjunctivitis. Subsequent studies, carried out in vivo by Dr. Daikos and myself,[6] yielded similar results.

In 1950, Dr. Ruchman requested us to send him this strain of virus inasmuch as he had been unable to obtain a strain of the epidemic keratoconjunctivitis virus of Sanders from other sources. In his hands the EK-TC 17 strain of virus yielded higher titers than those reported by previous investigators; when it was tested with antisera against various neurotropic viruses it was neutralized by St. Louis encephalitis antiserum and to a lesser degree by specific Japanese B and West Nile encephalitis antisera.[7] These results were confirmed in our laboratory.[8]

Following this Dr. Braley sent Dr. Ruchman another strain of epidemic keratoconjunctivitis virus. This, labeled by Dr. Braley EK-103 P.61 but referred to by Dr. Ruchman as the Braley strain, was believed to have been derived from the same pool of viruses prepared by Dr. Sanders in 1942 as was EK-TC 17. The former had been carried through at least 100 mouse passages. It was quickly shown by Dr. Ruchman[7] as well as by myself[8] to be closely related to St. Louis encephalitis virus. Systematic attempts were then carried out in both laboratories to determine the exact degree of this relationship. We were anxious to include in these tests one or more of the herpeslike virus strains isolated by Dr. Maumenee and his collaborators; unfortunately all their stocks of viruses and of specific antisera had been discarded during the Second World War. A third strain of presumed epidemic keratoconjunctivitis virus was furnished Dr. Ruchman by Dr. E. H. Lennette who in turn had received it from Dr. Sanders in 1942; this agent was demonstrated by Dr. Ruchman to be identical with herpes simplex virus.[9]

The results of the work carried out in the two laboratories[7-10] may be summarized as follows:

1. The two strains of epidemic keratocon-

junctivitis virus, EK-TC 17 and EK-103 P.61, appeared to be identical.

2. It was impossible to distinguish St. Louis encephalitis virus (Hubbard and Webster strains) from epidemic keratoconjunctivitis virus by the standard intracerebral neutralization test.

3. Cross immunity tests yielded evidence of a serologic relationship between the two agents, but the results were less clear-cut.

4. As expected, some cross reactions were demonstrated between epidemic keratoconjunctivitis virus and West Nile virus and Japanese B encephalitis virus.

5. Infectivity tests in various species gave somewhat different results with the two viruses: for example, following intracerebral inoculation epidemic keratoconjunctivitis virus caused a fatal encephalitis in rabbits and guinea pigs as compared to St. Louis encephalitis virus which caused no demonstrable clinical effects in rabbits and no more than a transient pyrexia in guinea pigs.

Hammon and Sather[11] have included epidemic keratoconjunctivitis virus in their studies of the St. Louis encephalitis group of agents. They have concluded that, although it is closely related to all the strains of St. Louis encephalitis virus tested, certain differences, such as greater virulence for some laboratory animals and greater antigenic potency, tend to set it apart from the heretofore recognized strains of St. Louis encephalitis virus. In the course of their studies they demonstrated an antigenic relationship between epidemic keratoconjunctivitis virus and Murray Valley encephalitis virus, as expected.

The points of similarity and dissimilarity of the two agents may now be compared. Both agents are essentially neurotropic, both are relatively small (less than 50 mμ in diameter), both produce pathologic changes in the brain of the intracerebrally injected mouse which are characterized by perivascular lymphocytic infiltration and the presence of small inflammatory foci and focal glial proliferation. Neither virus gives rise to perivascular demyelination. As regards host range both agents are pathogenic for mice and young rats; the epidemic keratoconjunctivitis virus of Sanders gives rise to a fatal infection in rabbits and guinea pigs following intracerebral inoculation, while St. Louis encephalitis virus produces but a mild or inapparent infection as a general rule. Monkeys may be infected with either agent by the intracerebral route of inoculation; both viruses may be propagated readily in the embryonated egg.

The two agents cannot be distinguished on the basis of the standard intracerebral inoculation test. Cross immunity experiments (the challenge of animals immunized against one virus by the other agent and vice versa) have yielded evidence suggesting that although the agents are antigenically related certain definite differences do exist. These differences are somewhat greater than those recognized as existing between the well-known strains of St. Louis encephalitis virus —for example, Webster, Hubbard, Winkler, and others.

One point remains unclear. The original epidemic keratoconjunctivitis group of viruses as described by Dr. Sanders[1] gave an LD_{50} end-point of approximately 10^{-6}. The results obtained with strain EK-TC 17 in Boston in 1947 were similar. In Dr. Ruchman's hands both strains (EK-TC 17 and EK-103 P.61) when titrated gave LD_{50} end-points of 10^{-8} or better[7]—a finding readily confirmed in our laboratory not only with material sent by Dr. Ruchman, but also with aliquots of EK-TC 17 which had been passed but once after storage in the solidified CO_2 cabinet since 1947.[8] In commenting on this point in 1951 Dr. Braley[4] stated that when the EK-TC 17 strain of virus was returned to him by the Upjohn Company in 1946 the LD_{50} end-point was found to be as high as 10^{-7}

Attempts were then made to investigate the relationship of the strains of epidemic keratoconjunctivitis virus (EK-TC 17 and EK-103 P.61) to the clinical disease de-

scribed and studied by Dr. Sanders and Dr. Braley in 1942. As previously mentioned apparently only these two strains had survived the war years, while no specific antisera were available from the New York studies. Due to the kindness of Dr. Sanders and Dr. Korns we were able, however, to obtain several specimens of human serum presumed to contain specific antibodies against the virus.

It will be recalled that in his original papers Dr. Sanders[1] described the inoculation of a human volunteer with the virus of epidemic keratoconjunctivitis by the ocular route. The volunteer (Dr. Sanders himself) developed what Dr. Thygeson described as a mild but characteristic case of the disease. A specimen of serum drawn just before the inoculation of the virus was devoid of demonstrable antibodies; a specimen obtained a month later gave an antibody titer of 1,000 neutralizing doses. Dr. Sanders furnished us with two specimens of his serum, one drawn in 1950, and one drawn in 1952. Both showed significant levels of antibody against both St. Louis encephalitis and epidemic keratoconjunctivitis viruses as follows:

Date serum drawn	*Log. Neutralization Index*	
	SLE*	EKC(EK-TC 17)
9/25/50	2.3	2.5
5/12/52	2.1	2.9

1950: Hubbard strain; 1952: Webster strain.

So far as he knew Dr. Sanders had never had any contact with St. Louis encephalitis virus either in the field or in the laboratory before the date the 1950 serum specimen was drawn.[12]

In addition, Dr. Sanders was able to establish contact with the patient E. L. from whom the original isolation of epidemic keratoconjunctivitis virus was made in 1942. Presumably E. L. had had no contact with St. Louis encephalitis virus from this date until the time 10 years later (1952) when he furnished a freshly drawn sample of his serum to Dr. Sanders for antibody studies.[12] When this was tested in our laboratory no neutralizing antibodies could be demonstrated against either St. Louis encephalitis (Webster) or epidemic keratoconjunctivitis (EK-TC 17) virus. Similar results were obtained by Dr. Ruchman.[13]

Two convalescent serum specimens from the Schenectady outbreak of 1943[14] were furnished us through the kindness of Dr. R. F. Korns, who is discussing the epidemiology of this outbreak on this program today. One specimen represented a pool of convalescent serum, obtained in 1943, from individuals all of whom had suffered clinically typical cases of epidemic keratoconjunctivitis. During convalescence but before being bled an undetermined number of these individuals had been hyperimmunized with epidemic keratoconjunctivitis virus vaccine. The second specimen represented a single bleeding of one patient (Crouch) who during convalescence from a typical attack of the disease had received several injections of the same vaccine before being bled as a source of hyperimmune serum. The results may be expressed in tabular form as follows:

Serum specimen log. neutralization index		
	SLE (Hubbard)	EKC (EK-TC 17)
Schenectady pool	1,000	< 10
Patient Crouch	100	< 10

It was surprising to say the least to find antibodies present against St. Louis encephalitis virus but not against epidemic keratoconjunctivitis virus.

Thus in summary[8] one may say:

1. Patient E. L. showed no antibodies against St. Louis encephalitis virus or epidemic keratoconjunctivitis virus 10 years following a "naturally" incurred attack of epidemic keratoconjunctivitis.

2. Patient M. S. showed moderate titers of antibodies against both St. Louis encephalitis and epidemic keratoconjunctivitis viruses 10 years following deliberate self-inoculation with epidemic keratoconjunctivitis virus.

3. Sera (one individual specimen and

one pooled specimen from several individuals) drawn in 1943 from patients who had recently recovered from attacks of epidemic keratoconjunctivitis during the Schenectady outbreak of that year and who had been hyperimmunized during convalescence with several injections of epidemic keratoconjunctivitis vaccine, showed moderate titers of antibody against St. Louis encephalitis virus but no demonstrable antibody against epidemic keratoconjunctivitis virus.

Since 1942 there have been numerous reports of outbreaks of epidemic keratoconjunctivitis in various parts of the world. The literature through 1952 has been reviewed by Cockburn,[15] who has carried out a number of epidemiologic investigations of outbreaks occurring in the western part of the United States. From three outbreaks we received eye scrapings and washings through the kindness of Dr. Cockburn and the Communicable Disease Center Virus Laboratory in Montgomery, Alabama. Attempts to isolate a viral agent by the inoculation of suckling and adult mice by various routes failed; in one instance tissue cultures were inoculated as well according to the technique described by Dr. Sanders; again the results were negative. Unfortunately these specimens arrived in poor condition, having thawed in transit to our laboratory from the Communicable Disease Center in Montgomery.

Cockburn and his associates[16] carried out a careful clinical and epidemiologic study of a small epidemic of typical epidemic keratoconjunctivitis which occurred in Kansas City in 1951. Antibody studies were carried out on convalescent serum specimens obtained from four patients approximately 11 months after the onset of illness. No antibodies against either St. Louis encephalitis virus (Webster strain) or epidemic keratoconjunctivitis virus (EK-TC 17 strain) could be demonstrated. Similar tests were run on convalescent serum specimens obtained from four patients involved in a later outbreak of epidemic keratoconjunctivitis. These sera were collected one to three months subsequent to the onset of illness. Three of the four specimens showed no antibody against epidemic keratoconjunctivitis virus; the fourth gave a neutralization index of 10 which placed it at the lower limit of the equivocal zone. One of the first three specimens showed a neutralization index of 125 against St. Louis encephalitis virus. The other three were negative. Inasmuch as serum surveys in this area have shown that up to 20 percent of the population have antibodies against St. Louis encephalitis virus this finding of one positive out of 10 could well have happened by chance. On the basis of these serologic studies it seems fair to assume that neither St. Louis encephalitis virus nor the epidemic keratoconjunctivitis virus of Sanders was the etiologic agent of the outbreaks studied.

There remains for discussion a brief consideration of several of the more important studies aimed at isolating the epidemic keratoconjunctivitis virus of Sanders from outbreaks of typical epidemic keratoconjunctivitis.

Braley,[17] in 1944, using the technique described by Sanders, isolated a viral agent from an outbreak of the disease. This could not be maintained in serial passage inasmuch as it failed to produce death in the inoculated mice with any degree of consistency. On the basis of cross immunity tests carried out in mice that had recovered from infection with this agent and in those that had recovered from infection with the epidemic keratoconjunctivitis virus of Sanders, it was concluded that the agents were probably similar. Braley[4] has reported the isolation of other strains of epidemic keratoconjunctivitis virus but these have not been made available for comparison.

Arakawa and his associates[18] have reported the isolation of two strains of virus from conjunctival scrapings and of six strains of virus from blood of patients suffering from epidemic keratoconjunctivitis. These agents were isolated by the intra-

cerebral inoculation of young mice followed by blind passage at four to seven day intervals. After three to 10 such blind passages signs of viral activity (cerebral irritation followed by paralysis and death) appeared three to six days following the injection of the passage material. No trouble was experienced in maintaining these strains in mice by intracerebral passage; all strains of the virus appeared to be antigenically related.

A rise in neutralizing antibody titer was demonstrated in the sera of 20 patients from whom acute and convalescent serum specimens were obtained. The injection of relatively large amounts of mouse passage virus into a monkey was followed by the development of fever and bilateral conjunctivitis seven to 10 days later; it was concluded, however, that the findings were not typical of the disease as seen in man.

One strain was propagated on the chorioallantoic membrane of the developing chick embryo but no specific lesions were described. The investigators reported that the mouse passage virus could be distinguished from "trachoma virus" on the basis of serologic (neutralization and complement fixation) tests, but apparently no further attempt was made to identify the agent. Its relationship to the epidemic keratoconjunctivitis virus of Sanders remains undetermined and the desirability of further studies aimed at delineating the characteristics of the viral agents isolated by Arkawa and his collaborators is obvious. It is impossible to rule out the possibility that this viral agent represented a latent murine virus which was uncovered by the process of blind passage.

Sezer[19] isolated a viral agent from four of 25 patients studied during the acute phase of the disease. Two isolations were made by the inoculation of conjunctival scrapings on to fragments of human cornea engrafted on the chorioallantoic membranes of embryonated eggs. One strain was isolated by the direct inoculation of the chorioallantoic membrane; this was lost after four passages. The fourth strain was isolated by the direct inoculation of mice by the intracerebral route. Three of these strains were carried in serial passage in both embryonated eggs and in mice. In the former they produced definite lesions on the chorioallantoic membrane and in the latter, a fatal encephalitis. No lesions were produced on the cornea of the rabbit. Antibodies were demonstrated in the convalescent sera of two patients tested. The relationship of this agent to the epidemic keratoconjunctivitis virus of Sanders remains to be determined. Unfortunately the strains of virus sent by Dr. Sezer to Dr. Ruchman proved to be inactive when examined on arrival.[18]

Ormsby and Fowle[20] studied 61 convalescent sera from an outbreak of epidemic keratoconjunctivitis occurring in Windsor, Ontario, in 1951, and eight convalescent sera from a similar outbreak in Toronto. No neutralizing antibodies could be demonstrated against the epidemic keratoconjunctivitis virus of Sanders. Isolation studies carried out in eight cases, employing conjunctival washings and scrapings for the inoculation of tissue cultures prepared according to the technique of Sanders, failed to yield evidence of the presence of epidemic keratoconjunctivitis (Sanders type) virus, although five strains of mouse-encephalomyelitis virus were isolated by the inoculation of serial passage "test" and "control" tissue culture material into mice. The rate of recovery was increased by administering cortisone to the mice prior to inoculation.

Hofman and Presinger[21] have reviewed the European literature and have presented the results of their own experiments. Filtrates of corneal scrapings obtained from patients suffering from clinically diagnosed cases of epidemic keratoconjunctivitis proved infectious for man when inoculated on the scarified cornea. The virus could be propagated in embryonated eggs only when Sezer's technique[19] was followed—namely, when conjunctival scrapings were inocu-

lated on to the chorioallantoic membrane grafted with human corneal tissue.

Following this, two strains of virus were propagated in series by straight-forward inoculation of the chorioallantoic membrane, with the production of lesions resembling those described by Sezer. Attempts to propagate the virus in tissue culture, employing embryonic mouse tissue according to the method of Sanders, failed until chick embryo extract was added to the medium. In contrast to the findings of Sanders[1] and of Sezer,[19] however, the strains isolated proved to be of low virulence for mice, killing the animals but irregularly following intracerebral inoculation. Attempts to increase the virulence of the virus by serial passage failed.

Corneal lesions were produced in 40 percent of rabbits and 70 percent of guinea pigs following inoculation of the scarified corneas of these animals; the resulting keratitis was mild in character. Conjunctival inoculation gave negative results. Neutralizing antibodies were demonstrated in the sera of patients convalescing from the disease according to the authors; the test system employed was the corneal inoculation of guinea pigs. The results as published were suggestive rather than conclusive.

Although no mention is made of the possibility that herpes simplex virus was involved in these infections the results of filtration and electron microscope studies make it seem unlikely that this agent was the cause of the disease. Suspensions of conjunctival scrapings remained infectious after filtration through a Seitz EK "Ultra cellafilter" of a pore size of 80 mmμ. The virus was held back by a filter of a pore size of 50 mmμ. Round particles of a size approximating 55 to 60 mmμ were seen with the electron microscope in preparations made from experimentally infected and subsequently enucleated human eyes, from infected tissue cultures, and from infected allantoic fluid. Since these materials were infectious as proven by the production of keratitis following inoculation of the scarified guinea pig cornea the authors postulated that these particles represented the virus agent itself. If so, its small size ruled out the possibility that it could be herpes simplex virus.

The relationship of this strain of virus to the epidemic keratoconjunctivitis virus of Sanders, the agents isolated by Arakawa et al., and to that described by Sezer remains undetermined. A number of other viral isolations have been reported by European investigators during the past 15 years; the significance of these reports cannot be determined until further studies are carried out. Particularly needed are (1) antibody studies, to determine the relationship of a given virus to the clinical disease and to determine the prevalence of infection with the agent, and (2) comparative studies (including antigenic analysis) of the various agents isolated in order to determine what relationship (if any) exists between themselves, and between them and recognized viral agents.

Finally it should be mentioned for the sake of completeness that two members of the APC group of viruses (types 3 and 8) have been isolated in recent months from cases clinically diagnosed as epidemic keratoconjunctivitis (Fowle et al.,[22] Jawetz et al.[23]). The significance of these findings is reviewed elsewhere in this program by Dr. Jawetz.

Summary

Two strains of the epidemic keratoconjunctivitis virus of Sanders have been studied. Both were obtained from Dr. Braley. Both were believed to have originated from the virus pool made by Sanders in 1942, but there is some doubt as to this point. The two strains appear to be identical.

The epidemic keratoconjunctivitis virus of Sanders, or perhaps more properly the Sanders-Braley agent, is closely related to St. Louis encephalitis virus. Although it falls into this group certain characteristics appear to set it apart from the better known strains (Webster, Winkler, Hubbard, and others).

As might be expected, some serologic overlapping has been demonstrated with West Nile, Japanese B encephalitis, and Murray Valley encephalitis viruses.

The relationship of this agent to the outbreaks of epidemic keratoconjunctivitis studied in 1942-1943 is shrouded in mystery. The loss of all other strains of the virus, and of all specific antisera, makes it unlikely that this point can ever be cleared up. It may be pointed out that:

1. The patient (E. L.) from whom the original isolation was made in 1942 showed no demonstrable antibodies against either St. Louis encephalitis virus or epidemic keratoconjunctivitis virus (Sanders) 10 years later.

2. The volunteer (M. S.) who was inoculated with the virus of epidemic keratoconjunctivitis in 1942 showed a titer of antibodies against both St. Louis encephalitis and epidemic keratoconjunctivitis viruses 10 years later.

3. One pooled serum specimen and one single serum specimen drawn from individuals in Schenectady in 1943 who during convalescence from the natural disease had been hyperimmunized with vaccine made from the epidemic keratoconjunctivitis virus of Sanders, showed significant levels of protective antibody against St. Louis encephalitis virus, but none against epidemic keratoconjunctivitis virus.

4. There is no definitive evidence that the epidemic keratoconjunctivitis virus of Sanders has been isolated since 1942, with the possible exception of Braley's report of 1944.

5. Antibody studies carried out with convalescent serum specimens obtained from recent outbreaks of the disease in the United States and Canada have yielded no evidence to suggest that the epidemic keratoconjunctivitis virus of Sanders was the etiologic agent causing these outbreaks.

University of Pittsburgh.

References

1. (a) Sanders, M.: Epidemic keratoconjunctivitis ("shipyard conjunctivitis") 1. Isolation of a virus. Arch. Ophth., **28**:581-586, 1942. (b) Sanders, M., and Alexander, R. C.: Epidemic keratoconjunctivitis: 1. Isolation and identification of a filterable virus. J. Exper. Med., **77**:71-96, 1943.

2. Heyl, J. T., Allen, H. F., and Cheever, F. S.: Quantitative assay of neutralizing antibody content of pools of gamma globulin from different sections of the United States against the viruses of herpes simplex, lymphocytic choriomeningitis, and epidemic keratoconjunctivitis. J. Immunol., **60**:37-45, 1948.

3. Maumenee, A. E., Hayes, G. S., and Hartman, T. L.: Isolation and identification of the causative agent in epidemic keratoconjunctivitis (superficial punctate keratitis) and herpetic keratoconjunctivitis. Am. J. Ophth., **28**:823-839, 1945.

4. Braley, A. E.: Personal communication.

5. Calkins, H. E., and Bond, G. C.: Adaptation of virus of epidemic keratoconjunctivitis to development in extra-embryonic fluids of chick embryo. Proc. Soc. Exper. Biol. & Med., **56**:46, 1944.

6. Cheever, F. S., and Daikos, G.: Studies on the protective effect of gamma globulin against herpes simplex infections in mice. J. Immunol., **65**:135-141, 1950.

7. Ruchman, I.: Relationship between epidemic keratoconjunctivitis and St. Louis encephalitis viruses. Proc. Soc. Exper. Biol. & Med., **77**:120-125, 1951.

8. Cheever, F. S.: A possible relationship between viruses of St. Louis encephalitis and epidemic keratoconjunctivitis. Proc. Soc. Exper. Biol. & Med., **77**:125-129, 1951.

9. Ruchman, I., and Daniels, W. F.: Possible relationships of epidemic keratoconjunctivitis virus to other viruses. Federation Proc., **11**:480, 1952.

10. Ruchman, I.: Cross-immunity tests in animals with epidemic keratoconjunctivitis and St. Louis encephalitis virus. Proc. Soc. Exper. Biol. & Med., **78**:483-486, 1951.

11. a. Hammon, W. McD., and Sather, G. E.: Personal communication; b. Sather, G. E., Miller, B. J., and Hammon, W. McD.: Comparative study of strains of St. Louis virus. Bact. Proc., 1953, p. 49.

12. Sanders, M.: Personal communication.

13. Ruchman, I.: Personal communication.

14. Korns, R. F., Sanders, M., and Alexander, R. C.: Epidemic keratoconjunctivitis: Correlation of epidemiologic data and results of serum-virus neutralization tests. Am. J. Pub. Health, **34**:567-571, 1944.

15. Cockburn, T. A.: The present status of epidemic keratoconjunctivitis. Am. J. Ophth., **38**:476-485, 1954.
16. Cockburn, T. A., Nitowsley, H., Robison, T., and Cheever, F. S.: Epidemic keratoconjunctivitis: A study of a small epidemic. Am. J. Ophth., **36**:1368-1372, 1953.
17. Braley, A. E.: Epidemic keratoconjunctivitis. Tr. Am. Acad. Ophth., 1944, pp. 153-174.
18. (a) Arakawa, S., Ohashi, T., Seki, T., Kitamura, O., Sakurabayashi, H., and Goto, T.: Experimental studies on epidemic conjunctivitis. Acta Soc. Ophth. Japan, **56**:367-371, 1950; (b) Experimental studies on epidemic conjunctivitis. Yokohama M. Bull., **6**:9-19, 1955.
19. Sezer, F. N.: Cultivation of virus of epidemic keratoconjunctivitis on chorioallantoic membrane of fertile egg. Arch. Ophth., **49**:293-302, 1953.
20. Ormsby, H. L., and Fowle, A. M. C.: Epidemic keratoconjunctivitis. Am. J. Ophth., **38**:490-495, 1954.
21. Hofman, H., and Presinger, M.: Experimental studies on the virus of epidemic keratoconjunctivitis. Ztschr. f. Hygiene & Infectionskrankh., **141**:180-196, 1955.
22. Fowle, A. M. C., Cockeram, A., and Ormsby, H. L.: Virus isolations from patients with keratoconjunctivitis. Am. J. Ophth., **40**:180-188 (Nov., Pt. II) 1955.
23. Jawetz, E., Kimura, S. J., Hanna, L., Coleman, V. C., Thygeson, P., and Nicholas, A. S.: Studies on the epidemiology of epidemic keratoconjunctivitis. Am. J. Ophth., **40**:200-209 (Nov., Pt. II) 1955.

THE ETIOLOGY OF EPIDEMIC KERATOCONJUNCTIVITIS*

E. Jawetz, M.D., P. Thygeson, M.D., L. Hanna, M.D.,
A. Nicholas, and S. J. Kimura, M.D.
San Francisco, California

According to the descriptions of competent clinicians and ophthalmologists all over the world, the disease epidemic keratoconjunctivitis is a well-defined clinical entity. In the sporadic form the diagnosis requires the presence of the typical round subepithelial corneal opacities, but during epidemic outbreaks a varying proportion of patients who escape corneal involvement have also been assigned this diagnosis. The incubation period (seven to 10 days), natural course, and epidemiologic behavior of the disease are so uniform that a single etiologic agent might well be suspected. Because of the clinical characteristics of the disease, the cytologic picture encountered, and the lack of association with any special kind of bacteria, a virus has been implicated as the probable etiologic agent for at least 25 years. Yet the nature of that virus is not established at present.[1]

There has been no lack of work directed at isolating and identifying a virus.[2,3] Nor is there a lack of positive results, each author firmly claiming that he has isolated "*The* virus of epidemic keratoconjunctivitis." Unfortunately there is no agreement whatever concerning the nature and behavior of these viruses and most investigators fail to confirm the findings of their colleagues. Some representative claims are reviewed in Dr. Cheever's paper in this volume. The divergence in experimental findings is so great that any attempt to reconcile them appears futile. In addition the reviewer is struck by deficiencies in many reported experimental protocols which suggest that some claims are unsupported by evidence.

It may be useful to list some criteria which must be fulfilled before a claim for the identification of *the* etiologic agent of a viral disease can be taken seriously:

A. The agent should be isolated repeatedly from typical cases of the illness but not from

* From the Departments of Microbiology and Ophthalmology, and the Francis I. Proctor Foundation for Research in Ophthalmology, University of California Medical Center. Supported in part by grants from the National Institutes of Health (B604) and Burroughs, Wellcome and Company.

normal persons. It should clearly be derived from the material secured from humans, not from experimental animals or tissues, or picked up "in passage."

B. During the typical illness patients should have a significant rise in neutralizing (or other) antibodies to the isolated virus. Such antibodies should be demonstrable in most patients convalescent from the disease, but should be absent from most persons of the same age groups and the same geographic location who have not had the illness.

C. Inoculation of the agent into suitable human volunteers should reproduce the illness.

D. The serologic pattern in the population should be compatible with the distribution of the agent and the epidemiology of the disease.

E. Last, but not least, the isolated agent should be sufficiently stable for survival and shipment, under suitable conditions, to other laboratories to permit comparative studies. Every effort should also be made to determine whether the isolated agent fits into a group or type of virus previously identified and studied.

Other criteria can be devised. However, unless the ones just listed are fulfilled, the minimum requirements have not been met and no claim should be made that the newly isolated agent is "the etiologic agent" of a given disease. A brief scanning of the claims reviewed in Dr. Cheever's paper forces one to the conclusion that the evidence for none of the viruses is acceptable to establish it as the etiologic agent of epidemic keratoconjunctivitis. Furthermore, most of the viruses (with the exception of herpes simplex which could not cause an epidemic disease in adults) are unfortunately not available for study. As described in Dr. Cheever's paper the widely accepted "virus of epidemic keratoconjunctivitis" of Sanders[4] is currently represented by strains which are variants of St. Louis encephalitis virus[5,6] and bear no evident relationship to the disease as it has occurred in America[1] or Europe[7] since 1951. Thus it appears appropriate to continue the search for agents which would fulfill the above listed criteria in epidemic keratoconjunctivitis.

In the course of virologic studies on various forms of keratoconjunctivitis, we isolated in 1955 in HeLa cell tissue culture a distinct—then new— type of adenovirus from a patient with typical, severe epidemic keratoconjunctivitis.[8,9] Subsequent studies indicated a significant association between that virus and the disease epidemic keratoconjunctivitis in various parts of the world. For this reason the evidence is summarized here that adenovirus (APC virus) type 8 has been regularly associated with clinical epidemic keratoconjunctivitis occurring between 1951 and 1955 in North America, Italy, Switzerland, and Japan, and may play a role in the etiology of the disease. It must be stressed that the evidence is not complete that adenovirus type 8 is *the* etiologic agent of epidemic keratoconjunctivitis. The material is presented in the hope that others may take advantage of any possible opportunity to settle the question.

To date only three strains of adenovirus type 8 have been isolated. One came from our patient with severe acute epidemic keratoconjunctivitis,[8,9] the second was isolated by Dr. Y. Mitsui from a patient with probable epidemic keratoconjunctivitis in Japan, and the third from the acutely inflamed eye of a small child in Saudi-Arabia.[10] The association of this virus with epidemic keratoconjunctivitis therefore is based principally on serologic studies in our laboratory and on volunteer inoculations performed by Dr. Y. Mitsui in Kumamoto, Japan. The serologic data have been published in detail[11] and can be summarized as follows:

1. Of 70 patients with the clinical diagnosis of epidemic keratoconjunctivitis in the United States, Canada, Switzerland, Italy, and Japan during 1951-55, 66 or 94.3 percent had neutralizing antibodies to adenovirus type 8 in a serum dilution of 1:10 or greater. Of 140 individuals from the same geographic areas and similar age groups who

did not have clinical epidemic keratoconjunctivitis, only 10 or 7.1 percent had such antibodies.

2. Adequate paired sera (acute and convalescent) were available from 17 patients with typical epidemic keratoconjunctivitis in Chicago, California, and Japan. Fifteen of these developed a fourfold or greater rise in neutralizing antibodies to adenovirus type 8 during their illness.

3. There was considerable variation in the highest titer of neutralizing antibodies reached (1:20-1:640) and in the rapidity of its rise. Neutralizing antibodies declined quite rapidly so that titers greater than 1:20 were not encountered later than two years after onset of acute epidemic keratoconjunctivitis. Thus a retrospective serologic survey beyond four years was not possible.

4. Patients with epidemic keratoconjunctivitis who exhibited a marked antibody titer rise against adenovirus type 8 did not have such titer rises for other types of adenovirus, for herpes simplex virus, or for the preserved "EK" strain of St. Louis encephalitis virus.[9, 11]

Recently we have had the opportunity to examine sera from patients in Vienna and Graz, Austria, who suffered from epidemic keratoconjunctivitis in 1952-53. These sera were collected in 1956 (that is, three years or more after onset of epidemic keratoconjunctivitis) through the kindness of Dr. R. Biehling, Dr. H. Hofmann, Dr. C. H. Kempe, and Dr. T. Moritsch, to whom we are greatly indebted. Control sera from the same geographic area were also made available. A few sera were toxic in tissue cultures and could not be examined satisfactorily. Neutralization tests with adenovirus type 8 on the remainder of the sera gave the following results:

Of 42 patients with the clinical diagnosis of epidemic keratoconjunctivitis, 22 or 52.4 percent had antibodies in a titer of 1:10 or greater. Of 30 control individuals only four or 13.3 percent had such antibodies. This difference has high statistical significance ($P = < 0.01$). If it is remembered that a long time elapsed between onset of disease and collection of sera, it can readily be understood that only slightly more than half of the epidemic keratoconjunctivitis patients had a significant antibody titer.[11] The remainder of the patients may well have possessed antibodies shortly after the disease but lost them subsequently. Taking into account the time lag, the frequency of significant antibody in the controls (13.3 percent) is far higher than encountered in North America (4.8 percent) but probably lower that in Japan (33.3 percent). It would be compatible with the occurrence of large epidemics in Austria since 1938, and an accompanying prevalence of asymptomatic infection with adenovirus type 8.

Dr. Y. Mitsui of the Department of Ophthalmology, Kumamoto Medical School, Kumamoto, Japan, has recently inoculated adenovirus type 8 into five human volunteers. One eye of each volunteer was inoculated with infected tissue culture fluid, the other eye with uninfected control fluid. Since epidemic keratoconjunctivitis occurs endemically in Japan, the volunteers were kept in strict isolation for 10 days preceding, and during, the trial. The details of the experiment and its results are described by Mitsui et al. in a separate paper in this volume.

Three of the volunteers developed entirely typical epidemic keratoconjunctivitis, and a fourth had severe conjunctivitis but no keratitis. All four had striking serologic responses, with a marked rise in titer of neutralizing antibodies to adenovirus type 8. The fifth had no clinical response to three successive inoculations, suggesting previously acquired immunity. This was supported by the presence of neutralizing antibodies in a titer of 1:20 prior to inoculation, and a lack of antibody titer rise subsequently. From the eyes of two of the volunteers with "takes," the virus was re-isolated. A typical response to inoculation with adenovirus type 8 is shown in Figure 1 compiled from clinical data kindly supplied by Dr. Y. Mitsui. It

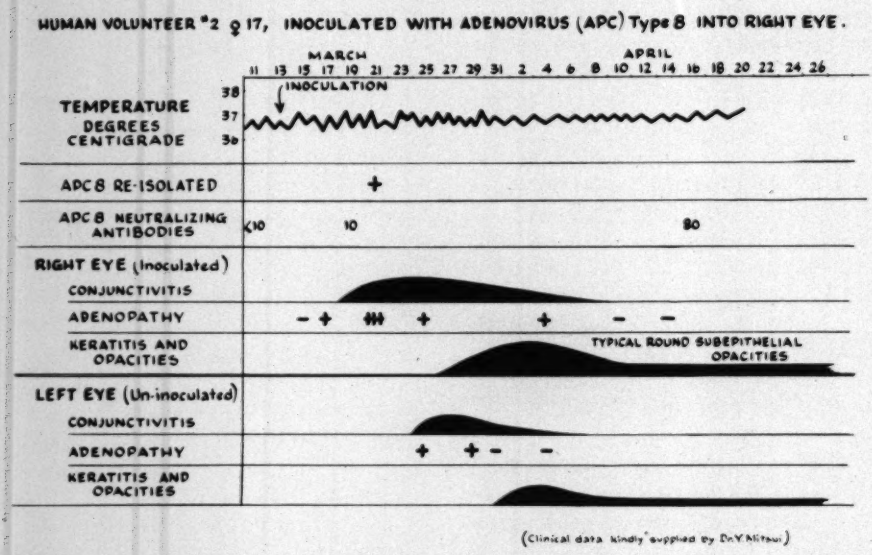

Fig. 1 (Jawetz, et al.). Laboratory and clinical findings in a volunteer inoculated with adenovirus type 8 in Japan by Dr. Y. Mitsui, who kindly made the clinical data available.

seems particularly significant that the infection spread spontaneously to the uninoculated eye.

In connection with one above-mentioned volunteer who developed conjunctivitis not followed by keratitis and a high titer of neutralizing antibodies to adenovirus type 8, another observation of Dr. Mitsui's deserves mention. He feels[12] that the infection (natural or artificial) which results in typical epidemic keratoconjunctivitis in adults, produces in children only membranous conjunctivitis and systemic symptoms but no keratitis. Paired sera from such a child were kindly made available to us by Dr. Mitsui. They showed an eightfold rise in neutralizing antibodies to adenovirus type 8 during the illness. This provides evidence that conjunctivitis without keratitis may be associated with infection by adenovirus type 8, if other persons in the area at the time suffer from typical epidemic keratoconjunctivitis.

Discussion

The evidence for the constant association of adenovirus type 8 with epidemic keratoconjunctivitis as it existed 1951-55 in North America, Italy, Switzerland, Japan, and Austria is convincing but incomplete. Additional virus isolations from typical cases, with complete serologic studies, are urgently needed. The human inoculation experiments of Dr. Mitsui indicate that infection of the eye with adenovirus type 8 in nonimmune individuals can result in a clinical picture indistinguishable from typical epidemic keratoconjunctivitis. However, it is not yet proven that adenovirus type 8, or a serologically closely related virus, is the *sole* etiologic agent responsible for this disease. It is, of course, possible that a variety of different agents may induce the sequence of reactions resulting in the clinical picture of epidemic keratoconjunctivitis and adenovirus type 8 may be only one of them. It is also possible that adenovirus type 8 acts as a precipitating agent but requires some other factor to produce the full clinical picture of epidemic keratoconjunctivitis. In this connection the repeated isolation of herpes simplex virus from epidemic keratoconjunctivitis must be mentioned.[9,13,14] This virus cannot be iso-

lated from normal eyes or from a variety of ocular inflammatory processes except herpetic keratoconjunctivitis (Hanna and Jawetz, this issue). Yet a number of observers have recovered it from acute epidemic keratoconjunctivitis. The significance of this observation is not clear at present but it deserves further study. The clinical differential diagnosis of epidemic keratoconjunctivitis includes active primary herpetic keratoconjunctivitis and pharyngoconjunctival fever caused by other types of adenovirus. Therefore, the isolation of these etiologic agents from some cases mistaken for epidemi keratoconjunctivitis may be expected.

Summary

An examination of past claims for the isolation of the etiologic agent of epidemic keratoconjunctivitis supports the conclusion that "there is at present no virus available that can be regarded with confidence as the etiologic agent of epidemic keratoconjunctivitis." Criteria have been listed which should be fulfilled before any claim for the isolation of an etiologic virus is made. Evidence has been presented implicating adenovirus type 8 in the etiology of epidemic keratoconjunctivitis, based on isolations, serologic data, and the results of volunteer inoculations with re-isolation of the virus and antibody response. The need for additional controlled observations has been stressed, and the possibility of multiple factors in the etiology of epidemic keratoconjunctivitis has been discussed.

University of California
Medical Center (22).

References

1. Cockburn, T. A.: The present status of epidemic keratoconjunctivitis. Am. J. Ophth., **38**:476-485 (Oct.) 1954.
2. Hogan, M. J., and Crawford, J. W.: Epidemic keratoconjunctivitis with a review of the literature and a report of 125 cases. Am. J. Ophth., **25**:1059-1078 (Sept.) 1942.
3. Hofmann, H., and Presinger, M.: Experimentelle Studien über das Virus der Keratoconjunctivitis epidemica. Ztschr. Hyg., **141**:180-196 (Apr.) 1955.
4. Sanders, M., and Alexander, R. C.: Epidemic keratoconjunctivitis: I. Isolation and identification of a filterable virus. J. Exper. Med., **77**:71-96 (Jan.) 1943.
5. Ruchman, I.: Relationship between epidemic keratoconjunctivitis and St. Louis encephalitis viruses. Proc. Soc. Exper. Biol. & Med., **77**:120-125, 1951.
6. Cheever, F. S.: A possible relationship between viruses of St. Louis encephalitis and epidemic keratoconjunctivitis. Proc. Soc. Biol. & Med., **77**:125-129 (May) 1951.
7. Pillat, A., Coronini, C., and Moritsch, H.: Zur Klinik und Aetiologie der Keratoconjunctivitis epidemica. Wien. klin. Wchnschr., **66**:798-801, 1954.
8. Jawetz E., Kimura, S., Nicholas, A. N., Thygeson, P., and Hanna, L.: New type of APC virus from epidemic keratoconjunctivitis. Science **122**:1190-1191 (Dec.) 1955.
9. Jawetz, E., Kimura, S. J., Hanna, L., Coleman, V. R., Thygeson, P., and Nicholas, A.: Studies on the etiology of epidemic keratoconjunctivitis. Am. J. Ophth., **40**:200-209 (Nov.) 1955.
10. Chang, R. S.: Personal communication.
11. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., and Kimura, S. J.: Antibodies to APC virus type 8 in epidemic keratoconjunctivitis. Proc. Soc. Exper. Biol. & Med., **92**:91-95, 1956.
12. Mitsui, Y., Tanaka, C., and Yamashita, K.: Change in the constitution with age: Its influence on the clinical symptoms of conjunctivitis. Am. J. Ophth., **39**:540-547 (Apr.) 1955.
13. Maumenee, A. L., Hayes, G. S., and Hartman, T. L.: Isolation and identification of the causative agent in epidemic keratoconjunctivitis (superficial punctate keratitis) and herpetic keratoconjunctivitis. Am. J. Ophth., **28**:823-839 (Aug.) 1945.
14. Moritsch, H.: Uebertragungsversuche mit dem virus der Keratokonjunctivitis. Zentralbl. Bakt., **160**:168-170, 1953.

EFFECT OF INOCULATING ADENOVIRUS (APC VIRUS) TYPE 8 INTO HUMAN VOLUNTEERS*

YUKIHIKO MITSUI, M.D., JUN HANABUSA, M.D., RYOJI MINODA, M.D., AND SHO OGATA, M.D.
Kumamoto, Japan

It has been found by Jawetz et al.[1] that a striking association exists between clinical epidemic keratoconjunctivitis and the development of antibodies to adenovirus type 8 (APC virus type 8). The etiologic role of this agent has not yet been established, however. The present study was undertaken in an effort to determine whether inoculation of adenovirus type 8 into the eyes of human volunteers would result in a clinical picture typical of epidemic keratoconjunctivitis. This paper reports the clinical observations on five human volunteers together with data on serologic responses and on the re-isolation of the infectious agent from the experimentally induced human disease.

MATERIALS AND METHOD

HUMAN VOLUNTEERS

At the time of writing, five Japanese volunteers have been inoculated and observations on four of them have been completed. There were two men and three women, aged 17 to 27 years. They had no history of acute conjunctivitis, no family history of acute conjunctivitis could be elicited from them, and examination failed to reveal any abnormalities in the conjunctival sac. No lesions could be found in the cornea by slitlamp microscopy.

VIRUS

A strain of adenovirus type 8 (Trim) in its 14th tissue culture passage was supplied by Dr. E. Jawetz, University of California Medical Center, San Francisco 22. This was sent by ordinary air mail, once in the form of a lyophilized, infected, HeLa cell tissue culture, and another time as a completely degenerated HeLa cell tissue culture in the liquid state. Both preparations were viable upon arrival and resulted in typical cytopathogenic effects when inoculated into washed HeLa cell cultures maintained in a medium of 10-percent chick serum in modified mixture 199 at 36°C. for four to seven days.

The fluid from several HeLa cell cultures which had undergone three-plus or four-plus degeneration after infection with adenovirus type 8 was harvested and centrifuged twice for 15 minutes at 3,000 rpm. The clear supernatant fluid served as infecting inoculum. Similarly prepared supernatant from uninfected HeLa cell cultures was instilled into the other eye to serve as control.

An attempt was made to re-isolate the virus from volunteers who developed manifest clinical signs of infection. On the second day of acute conjunctivitis, the conjunctiva was scraped by curette and the scrapings inoculated into fresh HeLa cell cultures. When degeneration appeared, the virus was passed in tissue culture. Re-isolated viruses were forwarded to Dr. Jawetz for identification by neutralization with specific immune rabbit sera.

SERA

A sample of sterile blood was obtained from each volunteer before inoculation and at intervals thereafter. After retraction of the clot, the sterile serum was separated and shipped to Dr. Jawetz in sealed glass vials. Neutralization tests were performed in his

* From the Department of Ophthalmology, Kumamoto University Medical School. Supported in part by a grant-in-aid from the National Council to Combat Blindness, Inc., New York. The serologic studies and identification of isolated viruses were performed by E. Jawetz and L. Hanna, University of California Medical Center, San Francisco. We are indebted to Dr. E. Jawetz and Dr. P. Thygeson for valuable suggestions and for aid in the preparation of this manuscript.

laboratory by techniques previously published,[1] and the results were reported as the reciprocal of the highest serum dilution resulting in significant neutralization of viral activity in tissue culture.

Inoculation procedure

The upper fornix of the conjunctiva was scraped lightly with a sharp knife under local anesthesia. The inoculum was then instilled into the conjunctival sac five times at five-minute intervals. After the last instillation a bandage was placed over the eyes. This was removed the next day.

In each volunteer the viral inoculum was inoculated into the right eye and the control inoculum into the left eye. Both eyes were inoculated simultaneously by two separate operators.

Observation of volunteers

The volunteers were hospitalized and placed in strict isolation for from three to 10 days prior to inoculation. After the inoculation they were inspected once a day in their rooms but were still strictly isolated until the onset of conjunctivitis. After that they were examined once a day by slitlamp and during this examination period had some contact with other patients. They remained in the hospital for one month or more after the onset of conjunctivitis. Bacteriologic and mycologic cultures of conjunctival material were made before inoculation and after the onset of conjunctivitis. Re-isolation of the virus after the onset of conjunctivitis was attempted in HeLa cells with material from all cases, and on rabbit cornea with material from Cases 3 and 5. The body temperature was measured twice a day during the period of hospitalization. For purposes of comparison the hematologic picture was followed in the last two cases.

Results

Case 1 (K. O., an 18-year-old male)

After three days' isolation, the volunteer was inoculated with the virus in the right eye and with the control materials in the left (March 2, 1956). The following day both conjunctivas were very slightly hyperemic and there was a scanty discharge. On the second day, however, this evidence of irritation had disappeared and the eyes remained normal until the eighth day after inoculation.

On the eighth day (March 9, 1956), the volunteer complained of slight discomfort in his right (virus-inoculated) eye. Inspection revealed the first signs of an acute conjunctivitis in this eye. It looked slightly follicular. The exudate was fibrinous and predominantly mononuclear. The conjunctivitis progressed rapidly and by the following day had become typically follicular. On the fourth day of the conjunctivitis the picture was that of a full-blown acute follicular conjunctivitis (fig. 1). Thin pseudomembranes tended to form when the conjunctiva was exposed repeatedly to the air by eversion of the lids for examination and photography. After seven days of activity the conjunctivitis began to subside slowly (March 16, 1956); about one month later the conjunctiva regained its normal appearance.

On the sixth day of the conjunctivitis in the right eye (March 15, 1956), a similar conjunctivitis developed spontaneously in the left eye. The clinical course of the conjunctivitis in this eye was slightly milder than that in the inoculated (right) eye.

Preauricular adenopathy. A slight pre-

Fig. 1 (Mitsui, et al.). *Case 1.* On the fourth day, a full-blown follicular conjunctivitis was present.

Fig. 2 (Mitsui, et al.). *Case 1.* On the fifth day, there were 13 distinct opacities.

auricular adenopathy appeared on both sides. On the right side the node became palpable two days after the onset of the conjunctivitis in the right eye and remained palpable for 10 days without becoming tender. On the left side it became palpable the day after the onset of conjunctivitis in the left eye. It was tender for the first several days and remained palpable for nearly three weeks.

Keratitis. On the eighth day of the conjunctivitis (March 16, 1956) there appeared a slight punctate keratitis in the right eye. The opacities increased in number and density during the first few days. On the fifth day (March 21, 1956) there were 13 distinct opacities and they were typically subepithelial (fig. 2). They were absorbed very slowly; one month later, distinct opacities, seven in number, were still demonstrable by slitlamp and three of them were grossly visible. It was about three months before the last opacity disappeared. In the left eye only a few corneal opacities appeared eight days after the onset of conjunctivitis in this eye. Ten days later they disappeared.

Miscellaneous findings. The volunteer did not complain of any systemic symptoms during the whole course of the disease, nor was there any indication of a systemic reaction in the temperature measurements taken. Bacteriologic and mycologic culture studies made before inoculation and after the onset of the conjunctivitis were essentially negative.

CASE 2 (H. I., a 20-year-old female)

After 10 days' isolation the volunteer was inoculated with the virus in the right eye and with the control material in the left eye as in the previous case (March 13, 1956). On the next day there was evidence of very slight irritation in both eyes but this had disappeared the following morning.

Four days after the inoculation the preauricular node on the right side swelled slightly. Two days thereafter an acute follicular conjunctivitis began in the right eye (March 19, 1956). The climax of the conjunctivitis was reached in three days when it appeared as a moderately severe acute follicular conjunctivitis with a tendency to form a slight pseudomembrane on repeated exposure of the conjunctiva to the air by eversion of the lids (fig. 3, second day; fig. 4, sixth day). Monocytes were predominant in the exudate. The climax lasted four days and then the conjunctivitis began to subside

Fig. 3 (Mitsui, et al.). *Case 2.* A tendency to form a slight pseudomembrane (second day).

Fig. 4 (Mitsui, et al.). *Case 2.* Pseudomembrane on the sixth day.

gradually. A little more than three weeks later it disappeared.

In the left eye a similar conjunctivitis appeared five days after the onset of the disease in the right eye. The course of the conjunctivitis in the left eye (spontaneously infected) was slightly milder than that in the right eye (virus inoculated).

Preauricular adenopathy. As already described, a swelling of the preauricular node appeared in the incubation period on the right side four days after the inoculation. It became slightly tender with the onset of the conjunctivitis. The tenderness disappeared in the course of several days but the node remained palpable until the 23rd day after the onset of the conjunctivitis. On the left side the node became palpable on the day of the onset of conjunctivitis and remained palpable for four days without becoming tender.

Keratitis. In the right eye a moderately severe punctate keratitis appeared eight days after the onset of conjunctivitis. The opacities increased in number and density during the following few days and the volunteer complained of a slight visual disturbance; the vision was 20/15 before the keratitis and 20/20 after its onset. It was typically subepithelial and 15 distinct opacities were counted six days after it began (fig. 5). Three weeks thereafter there were still nine opacities demonstrable by slitlamp, and four of the nine were grossly visible although the volunteer had regained a vision of 20/15.

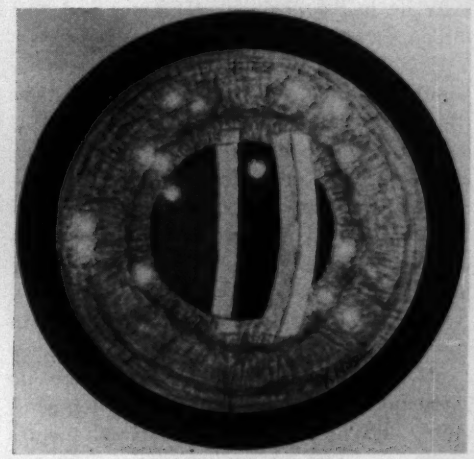

Fig. 5 (Mitsui, et al.). *Case 2.* Showing a typical subepithelial keratitis and 15 distinct opacities.

These opacities have persisted until the date of the latest examination August 15, 1956, about five months after the onset of conjunctivitis. Prof. G. Bietti, Rome, examined the volunteer on that date and found the picture typical of epidemic keratoconjunctivitis.

In the left eye there appeared a very slight punctate keratitis by the seventh day of the conjunctivitis. It did not interfere with vision although a few opacities have persisted until the latest examination.

Miscellaneous findings. The volunteer did not complain of any systemic symptoms during the whole course of the disease and there was no apparent rise in temperature. Bacteriologic and mycologic culture studies of the conjunctiva were essentially negative before inoculation and after the onset of conjunctivitis.

CASE 3 (W. K., a 17-year-old female)

After one week's isolation the volunteer was inoculated with the virus in the right eye and with control material in the left eye as in the previous cases (May 4, 1956). Seven days after the inoculation an acute follicular conjunctivitis began in the right (virus inoculated) eye (May 11, 1956). The

exudate had a fibrinous character and was predominantly mononuclear.

Six days later there was evidence of a similar conjunctivitis in the left eye. It took two and a half weeks to subside.

Preauricular adenopathy. A considerable swelling of the preauricular gland, with tenderness, was observed during the first week of the conjunctivitis on the right side. The adenopathy was very slight on the left side and lasted for a few days only.

Keratitis. Keratitis was not observed in this case during the course of the disease.

Miscellaneous findings. There were no systemic symptoms, no elevation of temperature, and no change in the blood picture with the onset of conjunctivitis. Bacteriologic and mycologic culture studies were essentially negative.

CASE 4 (Y. I., a 27-year-old female)

In this case the first inoculation with adenovirus type 8 was performed on May 4, 1956. On May 21, 1956, there had been no reaction, so a second inoculation was made on May 22, 1956. The result was again negative. A third inoculation was performed on June 1, 1956, and again there was no reaction.

CASE 5 (Y. M., a 27-year-old male)

After one week's isolation the volunteer was inoculated into both eyes with viral material (right) and control material (left) as in the previous cases (July 16, 1956). Five days later an acute follicular conjunctivitis began in the right, virus-inoculated eye (July 20, 1956). The conjunctivitis was rather moderate and lasted for three weeks (fig. 6, fifth day of conjunctivitis). The exudate was fibrinous and predominantly mononuclear. The second eye was not infected during the whole course of the disease.

Preauricular adenopathy. The adenopathy appeared on the day of the onset of the conjunctivitis. The node was palpable for three weeks without becoming tender.

Keratitis. On the ninth day of conjunctivitis there appeared a slight, but typical, subepithelial punctate keratitis (July 28, 1956). The opacities increased in number and four days later 17 could be counted (fig. 7, fifth day of keratitis). Professor Bietti, Rome, examined this volunteer on August 15, 1956, and found the opacities entirely typical of epidemic keratoconjunctivitis.

Fig. 6 (Mitsui, et al.). *Case 5.* Fifth day of conjunctivitis.

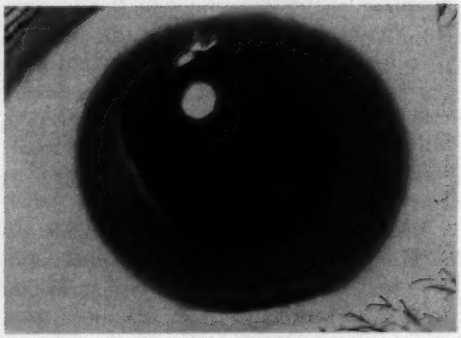

Fig. 7 (Mitsui, et al.). *Case 5.* Fifth day of keratitis.

Miscellaneous findings. There were no systemic symptoms and no temperature rise. Bacteriologic and mycologic culture studies were essentially negative.

LABORATORY OBSERVATIONS

The results of neutralization tests and virus isolations, in relation to the chronology of infection and clinical symptoms, are summarized in Table 1. It is seen that the first two volunteers had a striking rise in neutralizing antibodies while displaying clini-

TABLE 1

SEROLOGIC AND CLINICAL RESPONSE IN VOLUNTEERS INOCULATED WITH ADENOVIRUS TYPE 8

Volunteer	Day after Inoculation	Virus Re-isolated	Neutralizing Antibodies to Adeno-virus 8	Inoculated Right Eye		Left eye	
#1	0		<10	−	−	−	−
K.O.	3		<10	−	−	−	−
18	7		<10	+	−	−	−
male	13	−		++	+	+	−
	17			++	+	+	−
	21		20	++	+	+	+
	26			+	+	−	+
	46		80	−	+	−	+
	69			−	+	−	−
	106		80	−	−	−	−
#2	0		<10	−	−	−	−
H.I.	6		10	+	−	−	−
20	9	+		++	−	−	−
female	13			++	+	+	−
	29			+	++	+	+
	34		80	−	+	−	+
	52		40	−	+	−	+
	155			−	+	−	+
#3	0		10	−	−	−	−
W.K.	7			+	−	−	−
17	9	+		++	−	−	−
female	13			+	−	+	−
	24		80	−	−	−	−
	43		160	−	−	−	−
#4	0		20	−	−	−	−
Y.I.	3		10	−	−	−	−
27	17			−	−	−	−
female	0 (Re-inoc.)			−	−	−	−
	6		40	−	−	−	−
	9			−	−	−	−
	0 (Re-inoc.)			−	−	−	−
	16		20	−	−	−	−
#5	0		<10	−	−	−	−
Y.M.	4		10	+	−	−	−
27	9			+	+	−	−
male	15		20	+	+	−	−
	29		40	+	+	−	−
	44		40	−	+	−	−

Note: < before 10 indicates: "less than 10."

cally typical epidemic keratoconjunctivitis. The third volunteer had an equally marked rise in neutralizing antibody titer although her disease was limited to conjunctivitis, without the development of corneal lesions. This supports the belief of Mitsui et al.[3] that the same etiologic agent can produce typical epidemic keratoconjunctivitis in some individuals and only conjunctivitis in others.

The fourth volunteer failed to develop any clinical signs of infection during the first three inoculation attempts with virus grown in tissue culture. This would be compatible with at least partial immunity to the virus. It is therefore of great interest that serologic studies indicated the presence of significant specific antibody to adenovirus type 8 at the time of inoculation, without any definite subsequent rise in titer. The fifth volunteer developed a significant titer in specific antibodies during his illness which was typical of epidemic keratoconjunctivitis.

Re-isolation of the virus

Conjunctival scrapings were taken on the second day of the conjunctivitis from the first three volunteers. The material was suspended in a few drops of the maintenance medium with chick serum and inoculated directly into HeLa cell cultures.

The result with material from Case 1 was negative. A transferable, cytopathogenic agent was isolated from Cases 2 and 3. These agents were sent to Dr. Jawetz who found them to be adenovirus type 8 by neutralizing tests in tissue culture.

From Cases 3 and 5 scraped material was also inoculated into rabbit cornea to check the possible presence of herpetic virus. The results were negative.

The initial serum from all five volunteers contained a significant titer of neutralizing antibodies to herpes simplex virus. Each serum neutralized at least two logs of virus by intracerebral mouse neutralization test.

Discussion

The initial results of inoculation of adenovirus type 8 into five human volunteers have been encouraging. In three cases (1, 2, and 5) it resulted in an acute follicular conjunctivitis and preauricular adenopathy. The secretion was fibrinous and predominantly mononuclear. Subepithelial punctate keratitis typical of epidemic keratoconjunctivitis appeared about one week after the onset of conjunctivitis. The findings corresponded entirely to those of epidemic keratoconjunctivitis contracted by natural infection. A considerable rise in neutralizing antibodies against adenovirus type 8 was demonstrated in convalescent serum of these cases just as in spontaneously occurring epidemic keratoconjunctivitis.

In a fourth volunteer (3) the inoculation also resulted in an acute follicular conjunctivitis, but the symptoms and course of the disease were those of a mild epidemic keratoconjunctivitis without keratitis. In a fifth volunteer (4) repeated inoculations with adenovirus type 8 did not result in an onset of conjunctivitis. Serologic findings suggest a partial immunity in this patient.

The results in three of the five volunteers indicate that adenovirus type 8 is in all probablity the actual causative agent of epidemic keratoconjunctivitis, and the results in the fourth and fifth cases were not contradictory.

Evidently adenovirus type 8 cultivated in HeLa cells does not lose pathogenicity for human conjunctiva. It was our impression, however, that there might have been a slight lowering of virulence by cultivation. The clinical symptoms and the course of the disease in the four positive cases seemed to be slightly milder than in natural cases.

Summary

The initial clinical and laboratory results of inoculation of adenovirus type 8 into human volunteers have been presented. These initial findings are in keeping with the possibility that adenovirus type 8 may be the specific etiologic agent causing epidemic keratoconjunctivitis at the present time. Additional studies are under way to establish this impression more definitely.

Kumamoto University Medical School.

References

1. Jawetz, E., et al.: Studies on the etiology of epidemic keratoconjunctivitis. Am. J. Ophth., **40**:200-211 (Nov., Pt. II) 1955.

2. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., and Kimura, S.: Antibodies to APC virus type 8 in epidemic keratoconjunctivitis. Proc. Soc. Exper. Biol. & Med., **92**:91-95, 1956.

3. Mitsui, Y., Tanaka, C., and Yamashita, K.: Change in the constitution with age: Its influence on the clinical symptoms of conjunctivitis. Am. J. Ophth., **39**:540-547, 1955.

ISOLATION OF ADENOVIRUS TYPE 8 (APC TYPE 8) FROM A CASE OF EPIDEMIC KERATOCONJUNCTIVITIS IN JAPAN*

YUKIHIKO MITSUI, M.D.
Kumamoto, Japan
AND
ERNEST JAWETZ, M.D.
San Francisco, California

The first strain of adenovirus type 8 (APC 8) was isolated from the eye of a patient with acute epidemic keratoconjunctivitis.[1] Subsequent studies revealed a striking serologic relationship between the virus and the disease: A majority of patients with epidemic keratoconjunctivitis between 1951 and 1955 had antibodies to adenovirus type 8 and suitable paired sera showed a diagnostic rise in titer of neutralizing antibodies to this agent.[2] Mitsui et al. (this issue) inoculated human volunteers with adenovirus type 8 and demonstrated the development of an eye disease indistinguishable from typical epidemic keratoconjunctivitis. A second strain of adenovirus type 8 was recovered from a child with eye disease in Saudi-Arabia by Chang et al.[3] Further to support the suggestion that adenovirus type 8 may be involved in the etiology of epidemic keratoconjunctivitis, we wish to report an additional isolation of this virus from a sporadic case of epidemic keratoconjunctivitis in Japan.

CASE REPORTS

CASE 1

A four-year-old girl, the daughter of patient M. K. (Case 3) developed an acute conjunctivitis of the right eye in mid-April, 1956. Soon after the inception of the illness the child also had a fever and a slight diarrhea. The source of infection was unknown. Several days after the onset in the right eye, the left eye also developed a conjunctivitis. The illness was diagnosed by a physician as "membranous conjunctivitis."

The patient was seen in the eye clinic of Kumamoto University Medical School on May 8th, more than three weeks after the onset of the conjunctivitis. There remained only slight inflammation of the conjunctivas, with superficial cicatrization. The corneas were entirely clear.

CASE 2

A 58-year-old woman, grandmother of Case 1 and mother of patient M. K. (Case 3), developed an acute conjunctivitis of the right eye on April 29, 1956. She also had a mild headache but no other symptoms. About one week later she noticed an impairment of her vision.

When this patient was seen in the clinic on May 8th, there was a moderately severe acute follicular conjunctivitis of the right eye, with a tendency to capillary bleeding from the conjunctival surface. The right preauricular node was enlarged but not tender. The right cornea showed a moderately severe punctate keratitis. Some of these punctate lesions were slightly elevated above the surface of the cornea and these lesions stained with fluorescein; after a few days they flattened out and developed into the typical round subepithelial opacities of epidemic keratoconjunctivitis.

CASE 3

M. K., a 27-year-old woman, daughter of Case 2 and mother of Case 1, developed an intense conjunctivitis of the left eye on May 6, 1956. On that day she experienced a foreign body sensation in the involved left eye and noticed a slight discharge. On the

* From the Department of Ophthalmology, Kumamoto University Medical School, Kumamoto, Japan, and The Department of Microbiology, University of California Medical Center, San Francisco. Supported by grants from the National Council to Combat Blindness, Inc. (170-CO), and the National Institutes of Health (B 604).

TABLE 1

DEGREE OF CYTOPATHOGENIC EFFECT (+ TO ++++) OBSERVED ON SIXTH DAY OF INCUBATION

Serum Dilution	Virus Dilution (Trim)			Virus Dilution (Koyama)			
Antiserum to adenovirus 8	1:10	1:20	1:40	1:10	1:20	1:40	1:80
1:80	−	−	ND*	−	−	−	ND*
1:160	+++	−	ND*	++	−	−	ND*
1:320	++++	+	−	++++	+	+	−
1:640	++++	+++	−	++++	+++	+++	−
Normal rabbit serum 1:20	++++	++++	−	++++	++++	+++	−

* Not done.

next day the eye was deeply red and the lids were swollen, but there were no systemic symptoms or fever.

When this patient was seen in the eye clinic of Kumamoto University Medical School on May 8, 1956, there was a severe acute follicular conjunctivitis in the left eye. The discharge was fibrinous with a tendency to form a pseudomembrane. The cytologic picture showed a predominance of mononuclear cells in the exudate. Bacteriologic cultures were noncontributory. The left preauricular lymph node was markedly enlarged and tender. The cornea of the left eye was entirely uninvolved, and the right eye was normal.

This patient was seen at intervals in the clinic. The severe inflammation of the left conjunctiva lasted for one week, then subsided gradually so that only minimal signs were present after three weeks of observation. The cornea of the left eye remained clear and the right eye never became involved.

LABORATORY STUDIES

On May 8th, scrapings were obtained from the left conjunctiva of M. K. (Case 3) and were inoculated into twice-washed HeLa cell cultures in a maintenance medium of 10-percent chick serum in modified mixture 199 with added antibiotics. No early degeneration was observed in the inoculated cultures. The first evidence of a cytopathogenic effect occurred on June 2nd, more than three weeks after inoculation. The cytopathogenic effects were quite typical of those observed with adenoviruses. Degeneration of the initial cultures was complete on June 14, 1956. Repeated passages to new HeLa cell cultures were carried out after the beginning of cell degeneration.

The third passage of the virus and sera from the patient were sent from Kumamoto to San Francisco for additional laboratory study. The virus grew promptly in a fashion quite compatible with adenovirus type 8. It was completely neutralized by specific antiserum to adenovirus type 8 prepared in rabbits, but not by antisera to other types of adenovirus. An attempt was made to compare the behavior of this newly isolated strain (Koyama) with the established strain (Trim) of adenovirus type 8 by titrating both viruses in increasing dilutions against fixed dilutions of the same antiserum. The results of this "block titration" were as shown in Table 1. Apart from a slightly higher infective titer, strain Koyama appears to be indistinguishable from strain Trim.

Serial specimens of serum obtained from patient M. K. were examined for the presence of neutralizing antibodies, with the results shown in Table 2.

TABLE 2

SERUM STUDY OF PATIENT M. K.

Date Serum Obtained	Day after Onset	Dilution of Serum Capable of Neutralizing Adenovirus Type 8
May 8, 1956	3	<1:10
May 16, 1956	11	1:20
May 29, 1956	24	1:40

Discussion

The greater than fourfold rise in neutralizing antibody titer to adenovirus type 8 supports the impression of active infection by this agent (rather than a carrier stage) during the present disease. The question arises whether the new isolation could in fact have been a laboratory contamination of tissue culture by the adenovirus type 8. The facts militate strongly against such a possibility:

a. The initial incubation period until the appearance of cytopathogenic effects was three weeks. A limiting dilution of laboratory-propagated virus has, in our experience, never had such a long incubation period.

b. The newly isolated virus has a slightly higher infectious titer than the older strain.

c. The rate of antibody development in the patient makes it likely that she was infected with adenovirus type 8 before coming to the clinic.

d. Many other attempts in Kumamoto and San Francisco to isolate adenovirus type 8 have resulted in failure.

Thus it is concluded that another strain of typical adenovirus type 8 has been isolated from a sporadic case of epidemic keratoconjunctivitis. The diagnosis of epidemic keratoconjunctivitis without keratitis in patient M. K. is supported by the development of the fully typical disease with subepithelial opacities in her mother, and of the infantile nonkeratic form in her daughter.

Kumamoto University Medical School.
University of California (22).

Since this paper was written, three additional strains of adenovirus type 8 have been isolated from typical cases of epidemic keratoconjunctivitis in Japan.

References

1. Jawetz, E., Kimura, S., Nicholas, A. N., Thygeson, P., and Hanna, L.: New type of APC virus from epidemic keratoconjunctivitis. Science, **122**:1190-1191 (Dec. 16) 1955.
2. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., and Kimura, S.: Antibodies to APC virus type 8 in epidemic keratoconjunctivitis. Proc. Soc. Exper. Biol. & Med., **92**:91-95 (May) 1956.
3. Chang, R. S.: Personal communication.

CHARACTERISTICS OF HOSPITAL EPIDEMICS OF EPIDEMIC KERATOCONJUNCTIVITIS*

Irving H. Leopold, M.D.
Philadelphia, Pennsylvania

Outbreaks of epidemic keratoconjunctivitis in hospitals among patients attending eye clinics and among the physicians and nurses caring for these patients have been reported. In 1936, Hobson described 16 cases that occurred in staff patients of a Veterans' Administration hospital in San Fernando Valley, California. In 1951 Cockburn, Nitowsky, and Cheever reported on an epidemic in which nine patients had the infection, all of whom had attended a glaucoma clinic. This occurred in the Middle West. The latter epidemic apparently took place between May and July, 1951. The patients in the series (Cockburn et al.) had attended the glaucoma clinic for many years prior to the onset of this particular disease. No other cases appeared among the hundreds of patients examined in the eye clinic at the same time for other diseases. It was concluded, therefore, that the infection had taken place during an examination for glaucoma. There are many possibilities which are considered as the mechanism for infection, such as the fingers of the physician, the eyedropper,

* From the Wills Eye Hospital and the Graduate School of Medicine, University of Pennsylvania.

various instruments, towels, and the tonometer used for measuring intraocular pressure. It was the general conclusion of this study by Cockburn and his co-workers that the tonometer was the most likely vehicle of transmission. All other instruments, droppers, towels, and so forth, had been used by other patients who did not become infected.

There have been several recorded outbreaks in hospital clinics of Japan (Okamura and Mitsui, Aoki and Kasahara, and Mitsui and Tanaka). In the latter epidemic the topical anesthetic solution for tonometry was the iatrogenic source of infection.

Schneider, Kornzweig, and Feldstein described an outbreak of epidemic keratoconjunctivitis in a home for the aged. It was not possible to determine whether the transmission initially was through contaminated eyedrops or the tonometer. Subsequent cases were produced by eyedrop infection.

Two significant epidemics of keratoconjunctivitis occurred at the Wills Eye Hospital. The first was in 1950 during the months of December, January, and February. This was limited largely to patients who had the presenting symptoms on entrance to the clinics of the institution. This epidemic was associated with a widespread epidemic of this disease in the Philadelphia, Pennsylvania, and Camden, New Jersey, areas and was seen in other institutions throughout both cities. It was not possible to relate this epidemic to any particular hospital technique, instrument, or solution. The cases seen at the Wills Eye Hospital appeared to be part of a widespread incidence of the disease throughout the Philadephia area.

In 1953, however, there was a small epidemic involving chiefly the doctors and nurses of the hospital staff. At this time the Philadelphia area was relatively free of epidemic keratoconjunctivitis, but there was an epidemic prevalent in nearby Camden. There were 17 individuals associated with the hospital who developed the disease. Eight of these were residents in the hospital, two were nurses. One was an occupational therapist. Three were members of the attending staff, and two were patients who developed the disease during their hospitalization. Another patient was admitted with the disease, never having been to the hospital before. This patient had been admitted from a doctor's office undiagnosed, and the doctor who later diagnosed the condition developed the disease. The other two patients had retinal detachments and developed the disease during the period of their convalescence from the retinal surgery.

Clinical description

The conjunctivitis was severe. In all instances it was follicular in type. In practically all eyes there was marked edema of the conjunctiva. No false membranes were seen. There were two eyes, in two different individuals, which showed multiple subconjunctival hemorrhages. One doctor had petechial hemorrhages in the lids.

The discharge in all instances was slight, and in none did it appear purulent. Tearing and photophobia were prominent in all patients. In all, the preauricular glands were enlarged. All patients had bilateral signs of the disease, and the glands were enlarged on both sides. The second eye was usually less severely involved.

Nine patients developed corneal opacities. The corneal opacities were not large in these individuals but were numerous and persistent. They were nummular, appeared to be subepitheleal and beneath Bowman's membrane. In the majority of eyes the opacities disappeared in six months, the first physician involved in February, 1953, still has one corneal opacity after three years.

Cultures for bacterial growth were negative from all eyes. No inclusion bodies were seen in the conjunctival scrapings. Conjunctival scrapings showed a predominance of monocytes in the majority of the analyses, but polymorphonuclears were found in two patients on the first day of the disease. These

PATIENT	HOSPITAL ASSOCIATION	ONSET	CONVALESCENT SERUM	OPACITIES	ANTIBODIES WITH JAWETZ VIRUS
W.K.	Resident physician	February	O	X	Not Tested
E. R.	" "	"	O	X	
W. A.	" "	March	X	O	+
O. K.	" "	May	X	O	+
N. L.	" "	April	O	O	Not Tested
B. A.	" "	April	O	O	
F. M.	" "	April	O	X	+
W. F.	Attending Surgeon	April	X	X	Not Tested
W. R.	" "	March	O	X	
B. G.	Assistant Surgeon	April	X	O	+
M. S.	Nurse	March	O	X	+
E. D.	"	April	O	O	+
Mrs. E.W.	Patient	March	O	X	Not Tested
J. S.	"	April	X	O	
K. R.	"	April	X	O	
C. S.	Resident physician	June 6	O	X	
Mrs. C. S.	Occupational therapist	June 14	O	X	

Fig. 1 (Leopold). Findings in a hospital epidemic of epidemic keratoconjunctivitis.

became predominantly lymphocytic on the second and third days.

Duration of disease

The duration of the disease varied. In eight of the patients all symptoms of the disease disappeared within five weeks. In the nine with corneal opacities the duration varied. Symptoms were gone in approximately six weeks in four of these, and in the remaining five there were symptoms of photophobia persisting intermittently for six months. In only one of the patients, namely, W. K., is there still evidence of a corneal opacity.

Convalescent serum

Six of these individuals had convalescent serum taken from different physicians who had generally recovered from all symptoms of the disease. The serum was obtained 21 to 28 days after the first sign of the disease. Of those who received serum only one developed corneal opacities. Of the individuals who did not receive convalescent serum, totaling 11, eight developed corneal opacities. In all instances where the convalescent serum had been given, it was administered prior to the onset of any corneal lesion, within the first four days of the onset of the disease.

One of the residents who received serum, W. A., developed jaundice and impaired liver function. The serum had been administered intramuscularly to all either as 10 cc. on two successive days or 5.0 cc. twice daily on two successive days. The donor never had hepatitis and liver function tests were negative. The serum of W. A. was used for one other physician. He never developed any signs of liver disease.

Epidemiology

It has been known for many years that physicians themselves may transmit the infection from patient to patient in the course of eye examinations (Cockburn, Thygeson, Braley).

The possibilities of the source of infection are the fingers of the physician, various eye solutions, dropper bottles particularly, common instruments such as the tonometer, and ophthalmoscopes. It has been suggested that infective agents can survive for long periods in ophthalmic preparations. The tonometer has been incriminated frequently (Cockburn et al.).

In this small epidemic there was no patient who was contacted by all of the individuals who subsequently developed the disease. Patient E. W. was admitted with signs of the disease prior to the onset of corneal opacities and thus had the disease prior to hospital admission. This patient was examined by one of the physicians and one of the residents, but by none of the other sufferers. During the course of the examination the giant ophthalmoscope was used. This instrument was common to all of the physicians subsequently infected, with two possible exceptions, namely W. F. and C. S. Both of these physicians employed their own ophthalmoscopes and cannot recall having used the giant ophthalmoscope on the private floor. None of the other patients in the hospital on whom the tonometers of the private floor were used developed keratoconjunctivitis. None of the individuals in this series had a tonometer applied to his or her eyes.

Patients J. S. and K. R., admitted for retinal detachment, had been studied frequently by this giant ophthalmoscope. The epidemic keratoconjunctivitis did not appear until 10 and 14 days, respectively, after retinal detachment surgery. Each of these patients had his own bottle of atropine solution. They received no other local medication except anesthetics at the time of surgery. Other surgical patients who received these anesthetics did not develop the disease. Their eyes, however, had been examined by two of the residents who subsequently developed the condition. The attending surgeons in these cases never portrayed epidemic keratoconjunctivitis.

Two nurses who contracted the disease came in contact with the two patients who had the disease prior to the time that the diagnosis had been established.

W. K. was the resident who developed the disease first. He could not recall contacting a patient with this disease nor did he have any association with the patients who developed the disease. He had completed his duties as a resident at the hospital one week prior to the onset of his keratoconjunctivitis. The source of his infection is unknown.

C. S. developed the first signs of the disease on June 6th. The date is exact for this was his wedding day. He had completed his residency five days earlier. Mrs. C. S. had been in contact with the infected patients during her hospital duties as an occupational therapist. She did not develop the condition until seven or eight days after her wedding day, approximately June 14th.

The data suggest that the common instrument was the giant ophthalmoscope for the development in the other residents, as this was the only instrument which appeared to be common to all of the resident staff. Physician W. F. cannot recall having used this ophthalmoscope but could not be absolutely certain that he had not. It was his custom to use his own ophthalmoscope at all times. Although hundreds of hospital patients were handled by the involved residents during their incubation periods, no other patients ever demonstrated epidemic keratoconjunctivitis.

Antiserum

Six of these individuals could be contacted to obtain serum for testing against the virus (adenovirus type 8) isolated by Jawetz and co-workers. All six of these had neutralizing antibodies (Jawetz et al., 1955).

Summary

Seventeen individuals closely associated with one hospital developed signs and symptoms which were diagnosed as epidemic keratoconjunctivitis. Eight of these were resident physicians in the hospital. Three were attending physicians. One was an occupational therapist. Two of the group were nurses, and three were patients.

The source of the infection for the first resident infected in this group is not known. An instrument common to all but one of the other residents was the giant electric ophthalmoscope. None of these individuals had a tonometer used on his or her own eye. None had drops instilled into his eyes which might have been a common source of infection. The nurses may have developed the disease by handling the patients who subsequently developed epidemic keratoconjunctivitis.

All of the infected personnel showed bilateral disease, although the second eye was usually much less severely involved than the first. All cases had severe photophobia, tearing, and considerable discomfort. Foreign-body sensation was a very common complaint. All the patients had large preauricular glands. Nine of the 17 patients developed corneal opacities in one or both eyes.

In only one instance did other members of the family of each of the physicians, nurses, or patients, develop the disease. This spouse had been a member of the hospital staff.

Approximately 20 cc. of serum had been injected intramuscularly in six individuals. Only one of these developed corneal opacities and these were small and few in number and occurred in only one eye. One of these serum recipients developed reduced liver function and icterus.

1601 Spring Garden Street (30).

Acknowledgment

I am grateful to Miss Ada Padgett for considerable technical assistance.

References

Braley, A. E.: Annual review of the lids, lacrimal apparatus, and conjunctiva. Arch. Ophth., **51**:91, 1953.

Cockburn, T. A., and Danielson, R. W.: Present status of epidemic keratoconjunctivitis. Am. J. Ophth., **38**:476, 1954.

Cockburn, T. A., Robeson, T., Nitowsky, and Cheever, F. S.: Epidemic keratoconjunctivitis: The study of a small epidemic. Am. J. Ophth., **36**:1367, 1953.

Hobson, L. C.: Acute epidemic superficial punctate keratitis. Am. J. Ophth., **21**:1153, 1938.

Jawetz, E., Kimura, S. J., Hanna, M. A., Coleman, V. R., Thygeson, P., and Nicholas, A.: Studies on the etiology of epidemic keratoconjunctivitis. Am. J. Ophth., **40**:200 (Nov., Pt. II) 1955.

Thygeson, P.: The epidemiology of keratoconjunctivitis. Tr. Am. J. Ophth., **46**:366, 1948.

Mitsui, Y., and Tanaka, C.: Constitution and conjunctivitis. Acta Soc. Ophth. Japan, **54**:346, 1950.

Aoki, H., and Kasahara, C.: Epidemic keratoconjunctivitis. Acta. Soc. Ophth. Japan, **45**:573, 1941.

Okamura, K., and Mitsui, Y.: Diagnostic value of Prowasek's inclusion bodies in trachoma. Acta. Soc. Ophth. Japan, **45**:108, 1941.

Schneider, J., Kornzweig, A., and Feldstein, M.: Epidemic keratoconjunctivitis. Am. J. Ophth., **42**:267, 1956.

OFFICE AND DISPENSARY TRANSMISSIONS OF EPIDEMIC KERATOCONJUNCTIVITIS*

PHILLIPS THYGESON, M.D.
San Francisco, California

Epidemic keratoconjunctivitis, originally given the name of superficial punctate keratitis by Fuchs, was first defined as a clinical entity by the Austrian ophthalmologists, Adler and Fuchs, in 1889. The epidemic nature of the disease was recognized by these early workers but no mention was made of one of the principal features of the disease, namely its striking ability to transmit in doctors' offices, in dispensaries, and in hospitals. The disease has been known to be epidemic in the Orient, particularly in India, during the first half of this century and repeated epidemics in which many thousands of persons have been affected have been reported by Herbert, Wright, and others. Even in these studies little mention was made of the propensity of the disease to transmit in hospitals and offices.

The source of the American cases in 1941 and 1942 appears to have been an epidemic in Hawaii in which at least 25,000 persons were affected (Holmes). Holmes described in detail the characteristics of the disease but failed to mention its propensity for transmitting in offices and through medical personnel. Hogan and Crawford, however, in their first report of the American epidemic described its communicability in offices and in industrial dispensaries and mentioned the transmission of the disease to nurses and doctors.

My first experience with an office epidemic was in 1942 in New York when I was called in consultation in an office epidemic comprising some 80 cases. This office epidemic has been reported by Sanders and Forsheimer in detail. In 1948, I reported on the recurrence of epidemic keratoconjunctivitis in California during the preceding two years. A number of ophthalmologists in the western part of the United States were kind enough to give me detailed information in regard to cases occurring in their offices and I was thus able to study at first hand five distinct office epidemics. Following are some observations made on these outbreaks and others observed more recently.

FINGER TO EYE TRANSMISSION

This type of transmission has been most frequently encountered in the epidemics which I have been able to study and it is this form of transmission which I believe occurred in infections of 32 physicians and 40 nurses on whom I obtained data. In the course of the study observations were made on the surprising frequency with which the average person rubs his eyes. The rubbing of the eyes is done so commonly and unconsciously that the individuals under observation were surprised to have it called to their attention. The infectivity of the disease must of necessity be of a high order in view of the fact that the act of rubbing the eyes involves the skin only and not the ocular mucous membrane so that the virus must be carried to the conjunctiva by the tear meniscus. In our study of 33 doctors involved, only one recalls having had infectious material splash into his eye during treatment. There was general agreement among them that finger to eye transmission must have occurred.

If transmission of the infection could be carried from patient to doctor by means of fingers, it is obvious that it also could be carried from patient to patient by means of the doctors' fingers. No method has been available to determine the effects of ordinary hand washing with soap and water on con-

* From the Department of Ophthalmology and the Francis I. Proctor Foundation for Research in Ophthalmology, University of California School of Medicine.

tamination of fingers. It is of interest that in none of the office epidemics studied was there any other method of cleansing the hands than by the use of ordinary soap and water. The majority of the ophthalmologists and other medical men interviewed stated that they used ordinary hand washing between patients and it is to be presumed that with few exceptions this is a general custom. However, it can be categorically stated that such washing is generally of short duration and in no way resembles the surgical scrub required prior to surgery in hospital operating rooms.

Transmission by tonometers

There are now on record three well-studied epidemics originating in offices and dispensaries in which the tonometer has been incriminated as the transmitter of the disease. In one office epidemic of 19 cases which I had the privilege of studying, tonometry was used not only in glaucoma cases but also routinely in all refraction cases. In this office 14 infections developed the ninth day after tonometry, and it is of interest that in spite of precautions five secondary cases developed. It is possible that the minute epithelial abrasions produced by the tonometer may have been significant since all tonometry cases which I have studied have had keratitis, whereas keratitis is not an invariable feature of cases of epidemic keratoconjunctivitis contracted by other means.

The most recent study on transmission of epidemic keratoconjunctivitis by tonometry has been reported by Cockburn and associates. In this university glaucoma clinic transmission only occurred among glaucoma cases and all other patients remained unaffected.

Transmission by contaminated solutions

In a small office outbreak of 16 infections it was possible to pinpoint the source to a single case. In all of the 16 infections the only common factor was the administration of 0.5-percent pontocaine solution from a dropper bottle. It is believed that the nurse contaminated the dropper against the exciting patient's lid margin when administering the solution. All 16 cases were apparently contaminated on a single day since all developed infection nine to 10 days later. The five additional cases developed after another nine-day interval but neither pontocaine nor tonometry was concerned in these cases. It is believed that the hands of the attending ophthalmologist might have been responsible. It is interesting that there are only two secondary cases of transmission in the family arising from the 21 office infections which pinpoints again the importance of the ophthalmologist's office in the epidemiology of the disease. In the course of our study a number of other office infections were noted in which contamination of the dropper bottle may have been concerned in the infection but it was difficult to separate the various methods of transmission. Since at the time of the study there was no method of isolating the virus from contaminated solutions, survival time of the virus in such solutions could not be determined.

Other methods of transmission

During the course of the 1947-48 epidemic some 20 cases were seen in which the source of infection could not be determined. In the preceding period they had not been in attendance at any doctor's office or dispensary where they might have been accidentally exposed, nor were there any cases in their families, nor had they had contact with anyone to their knowledge with infected eyes. However, all of the cases had had multiple personal contacts. All were young, active adults who were in contact with numerous people in their work or in traveling to and from their work. None of these cases had had any trauma to their eyes nor any eye

irritation during the incubation period. Such cases suggest the possibility of nasal or pharyngeal carriers in the general population but this is purely speculative.

In view of the known propensity of at least two viral types of conjunctivitis or keratoconjunctivitis, namely inclusion and pharyngeal conjunctival fever, to transmit in swimming pools, a very definite attempt was made to elicit a history of swimming pool contamination in these cases. No such history could be obtained. There has been nothing about the epidemiology of epidemic keratoconjunctivitis to suggest that swimming pool transmission is of importance. There has been no summer incidence of the infection which is so characteristic of inclusion conjunctivitis and pharyngeal conjunctival fever, and a review of the literature has indicated no history of transmission of the disease in pools or lakes of any kind.

It is true that trauma of the cornea has been concerned in many of the office epidemics. Quite a number of the industrial cases seen in dispensaries have followed removal of foreign bodies and the disease has repeatedly occurred after minor operations on the eye, particularly after chalazion surgery and, as previously mentioned in some detail, after tonometry. That trauma is, however, not necessary for transmission of the disease is well known by the sources of the now very numerous infections in doctors and nurses who have been subject to no trauma, except that possibly concerned with the rubbing of the eyes with the fingers. It is of interest that the majority of the physicians concerned have been ophthalmologists, but a sizable body of general practitioners have been affected. Some of these have been workers in dispensaries where foreign bodies have been removed, whereas others have been engaged in general practice, some without any history of having taken care of inflamed eyes.

Whereas most of the minor epidemics have originated in doctors' offices or in dispensaries, other cases have developed in hospitals and in industrial concerns. In the shipyard epidemics contamination of welders' helmets and tools may have been a factor. It has been of interest that no epidemic has been reported to me in the United States with transmission in schools or in dormitories. In this respect, epidemic keratoconjunctivitis appears to differ decidedly from the pneumococcic and Koch-Weeks conjunctivitis which often spreads in the form of the well-known pink eye throughout schools. I have also been unable to find an instance of fly or gnat transmission such as is the case in the Middle East and also here in the United States in southeastern California and in the Rio Grande valley of Texas with Koch-Weeks conjunctivitis.

Epidemiologic control

The information regarding the epidemiology of the disease is admittedly incomplete due to lack of laboratory controls. Little can be said concerning the possibility of inapparent or subclinical infections or the possibility of respiratory carriers of the virus. Data derived from office infections, in some of which 100 percent of exposed individuals have developed the disease, certainly indicates that in the United States there is a high degree of susceptibility in the general population. This would certainly suggest that subclinical infections must be rare.

On the basis of experience gained in the 1941 and 1942 epidemics, a number of recommendations for the prevention of the disease were promulgated but unfortunately were not generally adopted. That these recommendations were, however, correct I think is shown by the experience in our own office in which a large number of epidemic keratoconjunctivitis cases have been seen in consultation and for purposes of special study without the occurrence of a single office transmission. The precautions taken in our office have been principally the meticulous washing of hands between examinations, using only soap and water, and the use of individual sterilized droppers for solutions.

All dropper bottles have been discarded as unsafe. All instruments, including tonometers, have been sterilized between patients. These simple precautions appear to have been effective.

Prevention of office and dispensary infections

Even in the incomplete knowledge which is now possessed regarding the nature of the virus of epidemic keratoconjunctivitis it would seem prevention of office infection depends primarily on dissemination of knowledge concerning the diagnosis of the disease and education leading to the common use of simple methods of prevention which should normally be a feature of any well-kept office.

Summary and Conclusions

Transmission of epidemic keratoconjunctivitis in offices and in dispensaries has been a major method of spread in the United States. Transmission of the disease is believed to have occurred following the use of contaminated tonometers, contaminated solutions, contaminated fingers, and possibly contaminated fomites—especially welders' masks, goggles, and common tools. Routine office and dispensary practice should be re-examined and the following prophylactic measures instituted, namely:

1. The discarding of all dropper bottles.
2. The use of individual sterilizable droppers.
3. Adequate hand washing with soap and water before and after treatments.
4. Sterilization of tonometers and all other instruments used on the eye.
5. The early recognition and isolation of cases.

University of California Medical Center (22).

A THEORY ON THE EPIDEMIOLOGY OF EPIDEMIC KERATOCONJUNCTIVITIS*

T. AIDAN COCKBURN, M.D.
Colombo, Ceylon

The theory presented is as follows:

In North America the causal agent of epidemic keratoconjunctivitis is a virus of the adenovirus group whose normal habitat is in the nasopharynx but which is capable of causing pathology in the conjunctiva and to a lesser extent in the cornea. When a susceptible eye is infected with this virus, the usual effect is the production of a relatively mild inflammation, but should conditions exist for the rapid passage of the pathogen from the infected eye to a series of susceptible eyes, then a strain of virus is produced that is much more specifically adapted to the superficial tissues of the eye. Infection with this virus strain produces the lesions of classical epidemic keratoconjunctivitis.

The difficulties in accounting for the permanent existence of the virus in North America purely on the basis of continued eye to eye transmission have been discussed in a previous paper.[1] In between major epidemics, cases of epidemic keratoconjunctivitis are extremely difficult to find, as those who have been trying to isolate the virus in the past few years are only too well aware. I myself have been searching for acute cases in the period 1953 to 1955 with the assistance of ophthalmologists in the midwest and eastern parts of the country with complete lack of success, and this has been much the finding of workers in the west as well. However, there were small outbreaks in Denver,[2] Chicago, and Philadelphia in that time. One concluded either that the infection was being imported from outside the country or else it existed somewhere in a masked form.

The possible connection of epidemic keratoconjunctivitis with some nasopharyngeal infection was first noted in 1951 when it was observed that the eye lesions of Greeley disease resembled mild cases of epidemic keratoconjunctivitis.[3] Greeley disease was an entity consisting of conjunctivitis, pharyngitis, muscle pain and pyrexia, and was later shown to be caused by infection with adenovirus type 3.[4] This observation has been confirmed in similar epidemics both in England[5] and Canada.[6] Inoculation of volunteers with either adenovirus type 3 or type 4 was found to produce a nonpurulent conjunctivitis with no corneal opacities,[7] but another similar experiment on a single volunteer did produce opacities.[6] Finally from an Asiatic sailor with epidemic keratoconjunctivitis on board ship in San Francisco, a virus was isolated that proved to be a member of the adenovirus group and which was designated as adenovirus type 8.[8]

It is interesting, however, that the typical severe illness of epidemic keratoconjunctivities has not been reproduced by inoculation of volunteers with an adenovirus and even if adenovirus type 8 virus were shown to cause such an illness, it would still be necessary to prove it to be the etiologic agent of the disease in North America as distinct from Asia and to explain where it is between epidemics. The second part of the theory, that a new strain of virus evolves in the course of direct eye to eye passage covers these points. The evidence in support of it is that in almost all outbreaks that have been adequately reported, it is specifically mentioned that the earliest cases to be found are mild, atypical, and often without opacities but that the later ones are much more severe and classical in form.

This pattern of disease was found in the following outbreaks:

In New York in 1942, when the first case "appeared to be a mild case without corneal

* From the World Health Organization.

involvement";[9] in a midwest city epidemic in 1951, when typical opacities "were found in eight of the nine cases, the only one not developing being the first case";[10] in a very large epidemic in the General Electric plant at Schenectady in 1942, when the early cases were described as mild and atypical;[11] in Denver, in 1953, when the initial patient had a chronic mild conjunctivitis for many months but developed the classical epidemic keratoconjunctivitis seven days after being treated with cortisone and then infected his wife and his physician.[2]

The experience of the epidemic in 1951 at the Ford works in Windsor, Canada, supports the theory.[12] "In March, 1951, however, at the infirmary in the Ford Motor Plant, the number of patients reporting with eye symptoms suddenly increased. These patients were complaining of a mild irritation in one or both eyes which were not thought to be other than the usual eye infections seen commonly in industrial practice.

"Between the second and third weeks following the appearance of the first cases, the nature of the disease changed abruptly. The onset of the symptoms was rapid and the course more acute. Corneal opacities occurred in the majority of the acute cases.

"As soon as it was evident that an epidemic of eye inflammation was in progress, certain measures were taken to prevent its spread from one individual to another.

"As the epidemic was subsiding in July, the eye infections were again much less severe and resembled those of the first patients encountered during May.

"The benign course of the first cases of epidemic keratoconjunctivitis occurring during the first three weeks of this epidemic and of those in the last month of the epidemic suggest a gradually increasing virulence in the virus as the epidemic progressed to its heights, with a subsequent decrease of virulence to the point where it was no longer infective."

This epidemic at Windsor is readily explainable much in the way as given by the authors of the paper quoted. There would be initially an outbreak of adenovirus infection as described for Greeley disease, with a number of patients suffering from conjunctivitis, then after a time some different eye-to-eye spread would lead to the appearance of an eye adapted strain producing more severe lesions. With the commencement of preventive measures this strain of virus would die out, leaving the milder form, which in turn would disappear as the whole population would be immunized by the airborne spread of the adenovirus. Incidentally, pharyngitis has not been described as a feature of epidemic keratoconjunctivitis but a virus adapted to the eye might well lose its virulence for the pharynx.

In areas such as Asia, where the hygienic conditions are such that direct eye to eye spread can be permanently maintained, the process of passage continued over many years could be expected to produce an organism varying significantly from the parent strain. It is therefore predicted that antigenic differences will be found between the epidemic keratoconjunctivitis viruses of North America and Asia.

c/o World Health Organization,
Box 1505.

References

1. Cockburn, T. A.: The present status of epidemic keratoconjunctivitis. Am. J. Ophth., **38**:476-485, 1954.
2. Cockburn, T. A., and Danielson, R. W.: Epidemic keratoconjunctivitis: Observations on four cases. Am. J. Ophth., **38**:486-490, 1954.
3. Cockburn, T. A.: An epidemic of conjunctivitis in Colorado associated with pharyngitis, muscle pain, and pyrexia. Am. J. Ophth., **36**:1534-1539, 1953.
4. Cockburn, T. A., Rowe, W. S., and Huebner, R. J.: Relationship of the 1951 outbreak of conjunctivitis and pharyngitis to type 3 APC virus infection. Am. J. Hyg., **63**:250-253, 1956.

5. Winning, J.: Epidemic keratoconjunctivitis in the Clydeside Area. Brit. J. Ophth., **40**:509, 1956.
6. Ormsby, H. L., Fowle, A. M. C., and Cockeram, A.: Epidemic keratoconjunctivitis and the APC virus syndrome. Canad. M. A. J., **73**:710-713, 1955.
7. Ward, T. G., Huebner, R. J., Rowe, W. P., Ryan, R. W., and Bell, J. A.: Pharyngoconjunctival fever in human volunteers inoculated with APC viruses. Science, **122**:1086-1087, 1955.
8. Jawetz, E., Kimura, S., Nicholas, A. N., Thygeson, P., and Hanna, L.: New type of APC virus from epidemic keratoconjunctivitis. Science, **122**:1190-1191, 1955.
9. Sanders, M., Gulliver, F. D., Forchheimer, L. L., and Alexander, R. C.: Epidemic keratoconjunctivitis, J.A.M.A., **121**:250-255, 1943.
10. Cockburn, T. A., Nitowsky, H., Robison, T., and Cheever, F. S.: Epidemic keratoconjunctivitis: A study of a small epidemic. Am. J. Ophth., **36**:1367-1372, 1953.
11. Perkins, J. E., Korns, R. F., and Westphal, R. S.: Epidemiology of epidemic keratoconjunctivitis. Am. J. Pub. Health, **33**:1187-1198 (Oct.) 1943.
12. McGavin, M. F., Boley, J. P., and Ormsby, H. L.: Epidemic keratoconjunctivitis. Canad. M. A. J., **69**:137-140, 1953.

ACUTE HERPETIC KERATOCONJUNCTIVITIS*

ALSON E. BRALEY, M.D.
Iowa City, Iowa

Clinically, the disease is similar to epidemic keratoconjunctivitis. There is edema of the conjunctiva, some chemosis, edema of the upper and lower lids, and a follicular conjunctivitis. The follicular conjunctivitis is frequently associated with the preauricular lymph adenopathy. The conjunctivitis is associated with either of two types of keratitis. During the first few days of illness, a diffuse epithelialitis is evident. At times, small dendritic figures may be present in various parts of the cornea. However, the dendritic figure is usually present in the palpebral fissure near the lower border of the cornea.

Maumenee, Hayes, and Hartman[1] reported an interesting group of cases of epidemic keratoconjunctivitis and herpetic keratoconjunctivitis in which they stated that herpes simplex virus can cause a keratoconjunctivitis which is clinically almost identical to that caused by epidemic keratoconjunctivitis. Maumenee et al.[1] reviewed the literature on acute herpes keratoconjunctivitis and found several cases reported of nonbacterial pseudomembranous conjunctivitis in which inoculation on a rabbit's cornea resulted in a "take" which was considered to be herpes-simplex virus.

Two of a total of six cases reported in the literature developed dendritic keratitis in the later stages of the disease. Gundersen,[2] in 1936, also listed four cases of conjunctivitis without keratitis due to herpes-simplex virus. Three of these patients had herpes of the lips. Maumenee et al.[1] state that one of the most helpful features, when it occurs, in differentiating the herpes conjunctivitis from epidemic keratoconjunctivitis is the presence of herpetic vesicles on the lips or lid margins.

In my experience, acute herpetic keratoconjunctivitis is a disease of young children and teen-agers. It usually begins as a unilateral acute conjunctivitis. The keratoconjunctivitis may occur alone as a follicular conjunctivitis associated with a preauricular lymph adenopathy and mild malaise. At times there are small vesicles on the cilia line which develop into small ulcers and which crust as healing occurs. The conjunctivitis may be associated with a local dermatitis of the eyelid, in which there are herpeta-form lesions of the upper lid and at times on the lower lid. In my experience, the skin of the upper lid is more frequently involved.

The keratoconjunctivitis is sometimes associated with herpes labialis and stomatitis. This is particularly true in infants and young children.

The conjunctivitis may be part of a generalized herpetic infection which may involve the face and occasionally other parts of the body. While the herpeta-form dermatitis associated with conjunctivitis appears to be most frequent in infants and young children, I was able to isolate herpes virus from vesicles and from the conjunctiva of a young woman in 1947.

This young lady was referred to me by Dr. Beatrice Keston because of her acute conjunctivitis. She had an extensive herpeta-form dermatitis of the face and upper trunk. I was able to remove the fluid from one of the vesicles in the skin, a herpes virus was isolated from the vesicular fluid and from scrapings from the conjunctiva. The keratitis has been the constant feature of all these cases.

The keratitis is usually diffuse during the

* From the Department of Ophthalmology, University of Iowa College of Medicine.

early phases of the disease but develops a dendritic pattern as the disease progresses. Even in the early phases, when there is a diffuse epithelialitis of the cornea, there is considerable loss of corneal sensitivity. This loss of corneal sensitivity is an important differentiating point between acute herpes keratoconjunctivitis and epidemic keratoconjunctivitis in which there is no loss of corneal sensitivity in the early phases of the disease. I think that acute herpetic keratoconjunctivitis is associated with edema of the lids and chemosis, and a follicular conjunctivitis with keratitis is probably a primary infection of herpes-simplex virus.

In the few cases where I have been able to measure the neutralizing substances present in the serum from patients with acute herpes keratoconjunctivitis, there has been a gradual rise in the titer of these circulating substances against a known strain of herpes-simplex virus. During the acute phase of the disease, there are no neutralizing substances, but subsequently neutralizing substances can be demonstrated in the serum.

Acute herpetic keratoconjunctivitis usually runs a course of from two to three weeks, but some keratitis may persist for several weeks after the acute phase of the disease has subsided. Fine opacities of the superficial cornea are not uncommon under what was the dendritic figure. Since those dendritic figures associated with keratoconjunctivitis are frequently in the lower half of the cornea, there is usually very little visual disturbance.

Summary

Acute herpetic keratoconjunctivitis may occur as a primary infection of the eye alone, associated with a stomatitis and/or labialis. It may occur as a part of a herpetic infection on the lids or of the face. It may occur as a part of a generalized hepatitis. When there is a well-developed dendritic figure on the cornea, the diagnosis is relatively simple. However, when there is a superficial keratitis, without any definite pattern, loss of corneal sensitivity may be an important diagnostic point.

I feel that an attempt to isolate the virus, either from the conjunctiva or from some of the vesicles on the lid border, should be made. Blood samples should be taken to demonstrate the presence or absence of neutralizing substances in the serum. If a rise in titer of these substances can be demonstrated during the disease process, then a presumptive diagnosis of herpes-simplex infection can be made.

University Hospitals.

References

1. Maumenee, A. E., Hayes, G. S., and Hartman, T. L.: Isolation and identification of the causative agent in epidemic keratoconjunctivitis (superficial punctate keratitis) and herpetic keratoconjunctivitis. Am. J. Ophth., **28**:823-839 (Aug.) 1945.

2. Gundersen, T.: Herpes cornea: With special reference to its treatment with strong solutions of iodine. Arch. Ophth., **15**:225, 1936.

SUPERFICIAL FORMS OF HERPETIC KERATITIS*

HUGH L. ORMSBY, M.D.
Toronto, Ontario

Herpes-simplex virus infections of the human cornea characteristically produce a branching or dendritic type of ulceration in the epithelium. In the early stages, a vesicle appears and enlarges in a series of branching processes to form the typical dendrite. The variations in the corneal picture, seen clinically, are due to such factors as the strain and virulence of the infecting agent, the degree of local immunity in the cornea, the existence of circulating humoral antibodies, the duration of the disease, the history of previous attacks in the same eye, the effect of treatment, and the presence or absence of secondary infecting agents.

A single vesicle may be studied histologically in the rabbits' cornea 24 to 36 hours after infection. There is a marked edema in the basal epithelial cells, extending to all superficial layers, and a grouping of nuclei to form giant cells. There is a tendency for the superficial cells to slough off. The typical Lipschütz body is a late development, and characteristically appears after 36 hours, forming a deeply staining eosinophilic intranuclear body. Smaller eosinophilic bodies, indicative of a response to trauma, may sometimes be seen in control scarified corneas in the basal layers of the epithelium. These must not be confused with the typical Lipschütz body. Stromal edema may be seen histologically after 72 hours, and new blood vessels begin to invade the cornea from this time onward in rabbits.

Clinically, the dendritic ulcer is typical in appearance and is easily recognized even by the inexperienced. Variations in the form of the ulcer occur frequently enough to bear description. In the event that treatment of the dendritic ulcer has been ineffective, or that the ulcer has not been recognized, it will usually continue to increase in size until large areas of the epithelium have become involved, and the "geographic ulcer" with uneven branching edges is formed.

Filaments of epithelium occur in corneas which have been subject to prolonged edema, or in ulcers which have been too frequently cauterized, resulting in poor adhesion between the basal layers and Bowman's membrane. These epithelial strands become drawn out by the movements of the eyelids, and become twisted upon themselves like rope.

Secondary bacterial infection rarely occurs in early dendritic ulceration of the cornea. However, in prolonged ulceration, secondary staphylococcic invasion of the ulcer bed may seriously hinder healing. For this reason, in long-standing ulcers of the cornea, antibiotics can often be used advantageously.

Marginal ulcers of the cornea normally result from a breakdown in the tissues at the limbus, secondary to bacterial infection of the lids or conjunctiva. Marginal ulcers, due to the virus of herpes simplex, occasionally occur peripherally near the limbus and may assume atypical characteristics. Due to the influence of humoral antibodies in the limbal vessels, the ulcer seldom extends as far as to the periphery, leaving a small ring of clear cornea. Central to this clear area, a deep chronic indolent nonbranching ulcer forms a gutter paralleling the limbus.

Superficial punctate erosions, occurring in herpetic keratitis, are also a manifestation of corneal malnutrition secondary to long-standing infection. However, in the earliest stage of acute primary ulceration due to the herpes-simplex virus, a number of small erosions, staining with fluorescein, may be seen with the slitlamp. Within 24 hours these develop into small dendritic figures.

Large recurrent erosions of the cornea, similarly, may occur in herpes simplex

* From the Department of Ophthalmology, Faculty of Medicine, University of Toronto.

corneae. They usually appear in healed lesions in which a weak adhesion exists between the epithelium and Bowman's membrane. Like the punctate erosions, they indicate an unstable epithelium but occur at the stage of healing or after healing has been long established.

Associated lesions

Follicular conjunctivitis is an invariable accompaniment of acute primary herpetic keratitis of children. In adults with circulating humoral antibodies to the virus, a follicular reaction seldom accompanies the dendritic ulceration.

Conjunctival ulcers, due to herpes-simplex virus, may be seen occasionally as a primary or secondary manifestation of dendritic keratitis. These ulcers of the conjunctiva are of short duration, healing spontaneously in a few days.

Herpetic ulcers of the eyelids and lid margins frequently precede the initial corneal infection in adults. Frequently there is a history of recurrent lesions in these areas over a period of many years before the cornea becomes involved.

Therapy

Unlike the adenoviruses, which are highly resistant to antiseptics, the herpes-simplex virus is readily destroyed by antiseptics such as iodine and ether. Thus, cauterization of a dendritic ulcer with iodine, together with denudation of the epithelium, is an effective method of treatment for the superficial lesions.

Following the introduction of the antibiotics, hope was initially held that these new agents might prove effective in the treatment of herpetic lesions. Early clinical trials with aureomycin (Braley and Sanders,[1] Thygeson and Hogan[2]) were thought to be favorable, but all authorities now agree that none of the antibiotics now in use has any effect on the course of a dendritic ulcer. Studies (in vitro) with one strain of virus in our hands[3] showed that the titer was lowered by aureomycin, in concentrations comparable to those obtained in the conjunctival sac during topical therapy. It is not thought likely, however, that intracellular virus is affected by the drug.

The role of immunity in virus infections of the cornea is of interest. In the rabbit, it has been shown in our laboratory[4] that following recovery from a primary herpetic keratitis, the same eye is relatively immune to a subsequent inoculation within a period of three months, whereas the second eye, upon inoculation, undergoes a typical primary reaction in spite of the presence of circulating antibodies. That these circulating antibodies play a small but important part in protecting the second eye was shown by the lessened conjunctival exudate and reduced residual scarring in this "protected" eye.

In further studies on corneal immunity[5] it was shown that upon hyperimmunization of the rabbit with intravenous vaccinia virus, titers up to 20 times those obtainable by skin or corneal vaccination resulted, but protection of the previously uninoculated cornea to an inoculation with the virus could not be obtained. On the other hand, subconjunctival vaccination with attenuated virus following primary skin vaccination resulted in complete protection of the cornea in a number of eyes upon inoculation with the virus three days after the last injection.

Therapy of herpes-simplex infections with corticosteroids has been shown by many workers to have a deleterious effect on the corneal lesions.[6,7]

Summary and Conclusions

1. The typical superficial form of herpetic keratitis is the dendritic ulcer. Variations in the typical pattern are due to the differences in the strain and virulence of the virus, the degree of local and humoral antibodies, the presence of secondary infection, and the type of therapy.

2. Associated herpetic ulcers of the skin of the face, lids, and lid-margins are commonly present and frequently precede or accompany the corneal infection.

3. Therapy with antibiotics is ineffective in herpes-simplex infections, whereas denudation of the epithelium followed by cauterization with iodine is usually effective. Hormonal therapy has a deleterious effect on the superficial herpetic lesions.

4. The presence of circulating antibodies modifies the degree of reaction in an eye with dendritic ulceration, but in the absence of vascularization of the cornea, does not overcome the infection or prevent its recurrence. Local tissue antibodies have been shown experimentally to play a significant role in recurrent infection of the cornea.

Banting Institute (5).

REFERENCES

1. Braley, A. E., and Sanders, M.: Aureomycin in ocular infections. J.A.M.A., **138**:426-427 (Oct.) 1948.
2. Thygeson, P., and Hogan, M. J.: Aureomycin in the treatment of herpes-simplex corneas. Am. J. Ophth., **33**:958-960, 1950.
3. MacKneson, R. G., and Ormsby, H. L.: The effect of the broad and medium-spectrum antibiotics on the virus of herpes-simplex. Am. J. Ophth., **39**:689-691 (May) 1955.
4. Hall, R. L., MacKneson, R. G., and Ormsby, H. L.: Studies of immunity in experimental herpetic keratitis in rabbits. Am. J. Ophth., **39**:226-233 (Feb., Pt. II) 1955.
5. Speakman, J. S., and Ormsby, H. L.: Studies in immunity in vaccinia keratitis in rabbits. Am. J. Ophth., **40**:194-197 (Nov. Pt. II) 1955.
6. Ormsby, H. L., Dempster, G., and Van Rooyen, C. E.: The effect of cortisone on experimentally induced herpetic keratitis of the rabbit. Am. J. Ophth., **34**:1687-1692 (Dec.) 1951.
7. Thygeson, P., Hogan, M. J., and Kimura, S. J.: Cortisone and hydrocortisone in ocular infections. Tr. Am. Acad. Ophth., Jan.-Feb., 1953, pp. 64-85.

DEEP FORMS OF HERPETIC KERATITIS*

PHILLIPS THYGESON, M.D., AND SAMUEL J. KIMURA, M.D.
San Francisco, California

It is well known that the typical herpetic lesion is epithelial. On the skin and mucous membranes it is always epithelial and scarring does not occur unless there is secondary infection with pyogenic bacteria. Exceptionally, however, deeper tissues may be involved in herpes-simplex infection, particularly in a primary attack. This is the case in the primary herpetic hepatitis[1] and herpetic encephalitis[2] that may occur in infants and children.

Although these disseminated forms may sometimes produce lethal tissue changes, cicatrization is not one of them. In contrast, scar formation is a principal, if not invariable sequela, of all corneal disease due to herpes-simplex virus, and it is this feature which makes herpetic keratitis, even in its most superficial form, definitely hazardous to vision.

Chronicity is another feature of herpetic infection which seems to develop only in the cornea; herpes-simplex lesions of the conjunctiva and lids, for example, are invariably of short duration and heal spontaneously.

No explanation has been offered for these two unique features of corneal herpes except that they may be related to the fact that the cornea is avascular and does not participate fully in the immune processes of the body as a whole. One can say that subepithelial involvement of the cornea is the rule, and that although it may be confined to

* From the Department of Ophthalmology and the Francis I. Proctor Foundation for Research in Ophthalmology, University of California School of Medicine.

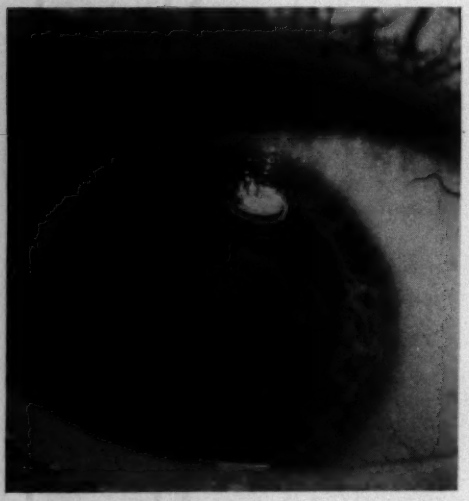

Fig. 1 (Thygeson and Kimura). Disciform keratitis.

the superficial subepithelial layer (Bowman's membrane), the stroma tends also to become involved; in a recently reported University of California series[3] there was stromal involvement in 59 of 200 cases.

Disciform keratitis

The classic example of deep involvement in herpetic keratitis is disciform keratitis (fig. 1), a viral lesion due principally to herpes-simplex virus but rarely to such other viruses as herpes-zoster virus, vaccinia virus, and the viruses of mumps and varicella. In the typical benign case, the lesion is characterized by an intense edema of the stromal fibers, limited principally to the central two thirds of the cornea and unaccompanied by any major necrosis of stromal fibers, or even by any new-vessel formation. This deep lesion may follow a clearly demonstrable superficial lesion such as the dendritic ulcer, or it may follow an insignificant epithelial lesion or one which occurred before the patient was first seen. Such benign cases tend to run a definite course of from two to three months and to heal with minimal scarring and little or no diminution of vision. This benign type of case was seen frequently in the prewar period but in our experience has become increasingly rare.

The more common type of disciform keratitis results in dense scar-formation, and sometimes in corneal necrosis with perforation. This severe form of the disease has been a feature of the postwar years and particularly of the period dating from the introduction of cortisone and the other steroids. Disciform keratitis now commonly follows an initial dendritic keratitis and is a dreaded development. Unlike the more benign prewar type of case, this severe type tends to last for many months, or even a year or longer, and to reduce vision seriously, often to light perception only. A most troublesome and painful complication is the development of secondary herpetic uveitis or iridocyclitis, often with secondary glaucoma. In this connection it should be mentioned that as a result of the characteristic corneal anesthesia, herpetic keratitis in the absence of uveitis or secondary glaucoma is relatively painless.

An almost invariable feature of this severe form of herpetic keratitis is a bullous keratopathy, and when the bullae rupture there is an exposure of the corneal stroma to secondary bacterial infection.

During the course of the keratitis program we have been conducting at the University of California during the past two years, we have seen in consultation a number of such cases of bullous keratopathy in which secondary bacterial or fungal infection has supervened. If the herpetic reaction is very severe and there is marked necrosis of the stroma, a deep herpetic ulcer results. Some of these lead to perforation and loss of the eye, or to the so-called "hypopyon keratitis" that will be discussed later.

Herpetic interstitial keratitis

As an extension of disciform keratitis, the entire corneal stroma may become involved. In our experience all such intersti-

tial cases have gone through a disciform stage. If this is the case and the patient is under continuous observation, the lesion cannot be confused with such other forms of interstitial keratitis as those due to congenital syphilis and tuberculosis. Herpetic sclerokeratitis and "herpes corneae posterior" have been referred to in the literature but have not been observed in our series. Since the clinical diagnosis of herpetic keratitis may be difficult unless a dendritic or disciform lesion has occurred, it seems to me that in some of the cases reported in the literature the diagnosis of herpetic etiology is open to question.

Hypopyon keratitis

Some of the dendritic ulcers observed in our series, particularly among those treated with steroids, have involved the anterior third of the cornea. Many of the so-called "geographic map" ulcers have been of this type. Deep ulceration, strictly comparable to the familiar deep central ulcer caused by various bacteria, particularly the pneumococcus, and by various fungi, particularly monilia, has also been seen as part of the herpetic keratitis picture. Of the 10 such ulcers observed in our series, six appeared to be due, at least in part, to secondary infection, and four to herpes-simplex virus alone. Hypopyon and severe uveitis have complicated both types, and both types have also been seen without hypopyon or uveitis.

The diagnosis of herpetic hypopyon keratitis has been simple when it has been possible to follow the case from the onset of a dendritic lesion, but late cases, seen in consultation only, have sometimes presented a diagnostic problem. In the absence of a virus isolation, two clinical features have been found to be particularly helpful diagnostically. These are:

1. The total anesthesia of the cornea, which is conspicuously uncharacteristic of any of the hypopyon ulcers due to bacteria or fungi.

2. The ulcer's retention, at least in part, of the characteristic ameboid or geographic map configuration (fig. 2).

Fig. 2 (Thygeson and Kimura). Herpetic hypopyon ulcer.

The examination of scrapings from these deep ulcers has not yielded any diagnostically significant cytologic features like the giant epithelial cells found in superficial herpetic ulcers, but direct scrapings have been very useful in determining the presence or absence of secondary bacterial infection. Cultures have of course also been taken routinely, but infecting organisms have always been demonstrable first in scrapings.

Herpetic iridocyclitis

A transient iris irritation (self-limited, accompanied by only a few cells in the aqueous beam, and with no keratic precipitates on the endothelium) is a common feature of the superficial form of herpetic keratitis. The iridocyclitis secondary to stromal forms of herpetic keratitis, however, is quite a different matter. It may be extremely severe and characterized by a circumcorneal flush, a heavy aqueous beam, many cells, and multiple keratic precipitates of medium size.

The cases in our series have shown a marked tendency to synechia formation and to secondary lens opacification. In about half the cases a rise in intraocular pressure has been noted. In a few instances we have been

able to study the aqueous obtained by puncture, but no herpes-simplex virus has been isolated. In spite of this failure, we believe that the iridocyclitis represents an extension of the virus into the uveal tissue since it tends to persist for some time after the original corneal lesions have healed.

As already mentioned, the severe pain that characterizes herpetic uveitis is in sharp contrast to the lack of pain associated with the pure keratitis. The severity of the iridocyclitis observed in our series has seemed to warrant our referring to it as a definite entity, that is, "herpetic kerato-uveitis." It is of interest that topical administration of the steroid hormones, which in herpetic keratitis has markedly suppressed the inflammatory signs, has had little suppressive effect on either the inflammation or the pain of the iridocyclitis. As will be discussed later, a high incidence of the unfavorable effect of steroid therapy on the clinical course of all types of herpetic corneal lesions has been noted in our series.

In the course of our studies of herpetic keratitis in the postwar years, we have in a number of cases observed the development of an iridocyclitis, without keratitis, in an eye previously subject to recurrent dendritic keratitis. Material from these cases has not been available for study, but the occurrence of such an iridocyclitis following fever or some other typical trigger mechanism warrants the conjecture that it may represent simply a recurrence of the disease in deep tissue, a part of the whole herpetic recurrence pattern. In the absence of a previous herpetic lesion of the cornea, however, we never have seen an iridocyclitis which we could regard as herpetic. In the literature there are claims that herpetic uveitis can occur in the absence of any previous corneal disease. Unfortunately the diagnosis of herpetic uveitis must be presumptive at best since iris tissue cannot be examined and since aqueous punctates are notoriously unsatisfactory for demonstrating microbial agents in any type of uveitis.

Relation of use of steroid hormones to development of deep forms of herpetic keratitis and uveitis

The striking suppressive effect of topical steroid therapy on the inflammatory phases of herpetic keratitis led to an early unwarranted enthusiasm for this form of treatment. This suppressive treatment was also popular with the patients, and once the steroids had been used it was almost impossible to wean the patients from them. It was soon recognized,[4] however, that these preparations had a masking effect which in some instances led to deep extension of the disease with ultimate loss of the eyes. A number of observers have reported perforations of the cornea in perfectly white eyes that were undergoing topical steroid therapy.

As a result of my own unhappy experience, I have been forced to conclude that topical cortisone therapy often increases the chronicity of the disease, the necrosis of stromal fibers, and the incidence of uveal complications (fig. 3). Regrettably, perhaps, this adverse effect of the steroid has not been uniform: I have records of four cases of dendritic keratitis that healed while the patients were receiving topical cortisone therapy.

It is a fact, however, that in our series all the severe cases of herpetic keratitis with

Fig. 3 (Thygeson and Kimura). Extensive corneal scarring in a case of herpetic disciform keratitis receiving extensive topical steroid therapy.

deep involvement and uveitis have been under steroid therapy at one time or another in the course of the disease. Perhaps it is unfair to blame the steroids for the greatly increased severity and frequency of these deep forms in the postwar years but there is certainly experimental evidence, as Dr. Kimura will report, that herpetic keratitis in the rabbit is worsened by topical steroid therapy.

Summary and Conclusions

The deep forms of herpetic keratitis and kerato-uveitis encountered in our keratitis series at the University of California are described. An increase in the severity and frequency of the deep forms in the postwar years has occurred concomitantly with the use of topical steroid hormone therapy. It is believed that the steroids have had only a masking effect on the disease and that they are at least partially responsible for the increased incidence of deep forms and visual loss. The nature of herpetic iridocyclitis and the possibility that an herpetic iridocyclitis may occur without a keratitis are examined.

University of California
Medical Center (22).

References

1. Zuelzer, W. W., and Stulberg, C. S.: Herpes simplex virus as the cause of fulminating visceral disease and hepatitis in infancy. Am. J. Dis. Child., **83**:421, 1952.
2. Scott, T. F. McN.: Diseases caused by the virus of herpes simplex. In Rivers, T. M.: Viral and Rickettsial Infections of Man, Lippincott, Philadelphia, 1952, ed. 2.
3. Thygeson, P., Kimura, S. J., and Hogan, M. J.: Observations on herpetic keratitis and keratoconjunctivitis. Arch. Ophth., **56**:375-388 (Sept.) 1956.
4. Thygeson, P.: Herpes corneae (editorial). Am. J. Ophth., **36**:269, 1953.

THE ETIOLOGIC ROLE OF THE VIRUS OF HERPES SIMPLEX IN OPHTHALMIC DISEASE

Geoffrey W. Rake, M.B.*
New York, New York

In order to survey the role which the virus of herpes simplex may play in human ophthalmic disease, it would seem best to begin by reviewing the natural history of the infection of man with this agent. One should, however, stress that herpes corneae is probably the most important keratitis which we are likely to encounter in this country.

The virus itself is, over a period of time, to be found almost ubiquitously in man and his excretions. So much so is this the case that over 60 percent of individuals have been infected by the age of five years and over 90 percent by the age of 15 years.[1] In by far the larger number of cases, over 99 percent, these primary infections are silent and can be detected only by a rise in neutralizing antibodies[2] and by the almost universal tendency for the individual now to carry the virus in the tissues and to suffer from attacks of recurrent herpes or fever blisters (fig. 1). This high infectivity but low patent morbidity suggests that herpes simplex may be an ancient infection of mankind in which the virus has attained an almost perfect symbiosis for its primary host, although still highly pathogenic for other species.[3] In this is may resemble B. virus in monkeys and pseudorabies in swine.

Not in every case, however, is this har-

* Research Professor, Microbiology in Medicine, University of Pennsylvania, Philadelphia; Scientific Director, International Division, Olin Mathieson Chemical Corporation, New York.

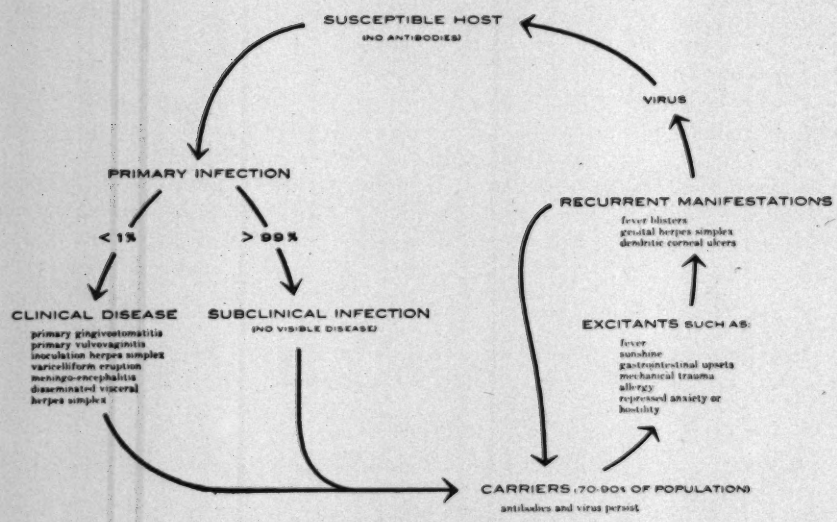

Fig. 1 (Rake). Host-parasite relationship of herpes-simplex virus in man. (From Blank and Rake, 1955.)

monious relationship so painlessly established. In perhaps one case in 500 the primary invasion of the body by the virus is accompanied by disease. In our experience this primary disease is usually what is known as acute herpetic gingivostomatitis—an acute and often serious disease with malaise, fever, and regional lymphadenopathy. It may, however, assume one of many other forms, including ophthalmic disease.

Now, although, because of the high prevalance of maternal neutralizing antibodies for the virus, most children are born with such antibodies, these disappear in the next six months and from then until the age of two years the presence of specific antiherpetic antibodies is unusual (fig. 2). This and the immediately following period of life is the time of greatest susceptibility and the time at which the manifestation of primary infection, whether it be Kaposi's eruption, gingivostomatitis, or acute keratoconjunctivitis, can be expected to occur. At the beginning of the infection, then, herpes neutralizing antibodies are absent; but they appear within five to 10 days unless the disease progresses rapidly to a fatal outcome. The skin test[4] also becomes positive; both are diagnostic of the nature of the primary infection.

In rare cases, perhaps because of low maternal antibodies or of a high dose of virus or of prematurity (and therefore greater susceptibility, the immature cells of all species being notoriously more vulnerable) or a combination of any two or all three, infection of the infant may occur during birth. In such cases the mother usually has recurrent herpes on the vulva or elsewhere around the genitalia. The disease usually commences on the skin, mucous membranes, or in the eye, and then becomes systemic and severe, not infrequently progressing to death. In the present instance the eye lesions, which may occur on the cornea, the conjunctiva, or the lids, are incidental and usually minor in the over-all picture and will not be discussed in greater detail.

Mention has already been made of the ubiquitous nature of the virus and this obviously plays an important role in the natural history. Not only do the majority of persons carry the virus continuously, if latently, in the tissues, but they suffer from

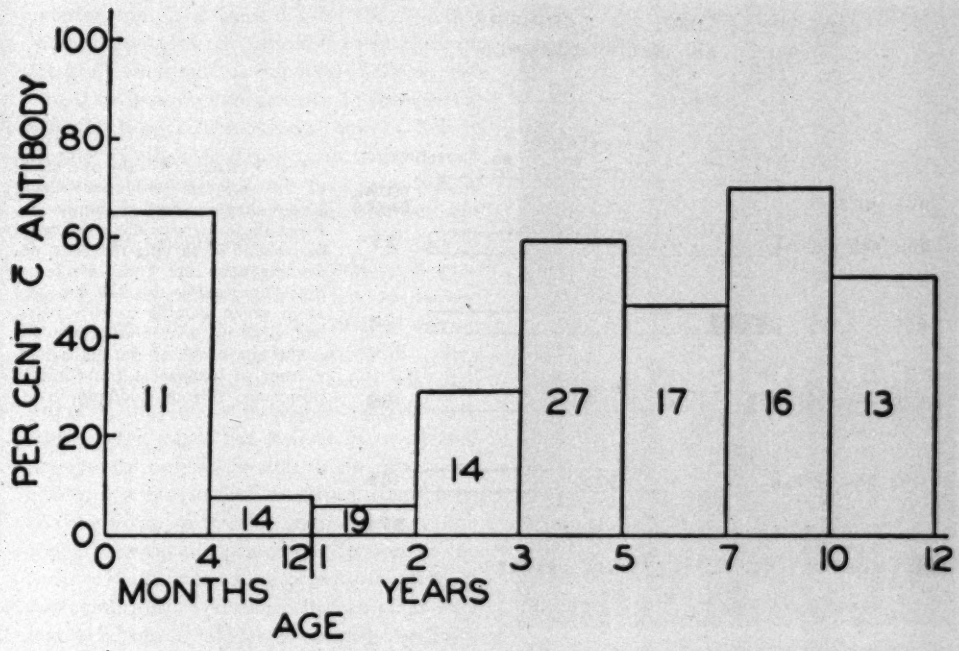

Fig. 2 (Rake). Incidence of herpes-neutralizing antibodies in the blood of 131 children. Numbers within each column indicate the number of children tested at each age. (From Scott et al., 1953.)

frequent attacks of fever blisters during which the virus is plentifully present in the open lesions. Even more important, the virus is present in the nasal and oral secretions or stools of 20 percent of apparently normal children and only less frequently in adults (fig. 3). It is of little wonder, therefore, that a high rate of infection is maintained constantly in such civilized communities as have been examined.

The nature of the agent, as it is carried in the tissues between attacks of recurrent herpes or fever blisters, is unknown. Blank and Rake[2] have suggested a condition akin to lysogeny in bacteriophage, but repeated careful attempts to demonstrate this experimentally have been unavailing.[5] In most individuals there will be a particular zone of the body, most frequently on the face but also around the genitalia, and not uncommonly on the cornea or conjunctiva, in which the recurrent lesions will occur. Any of several noxious stimuli, chemical or physical, or even mental stress, will cause a recurrence, and a given individual is liable to have a particular trigger to which he or she is responsive. These recurrent attacks may occur in the area of the primary manifestation when such occurred, but in any case the vesicles are smaller and the whole attack less severe than in the primary infection.

These recurrent attacks do not change the level of neutralizing antibodies which tend to be as high before the episode as after[6] (fig. 4). Virus can be isolated from the lesions and that it maintains its full virulence for its natural host is shown by such examples as those in which an infant is infected during birth from herpetic lesions around the maternal genitalia and frank and severe primary infection ensues. It is, therefore, not only the nature and perhaps the whereabouts of the virus between attacks which is puzzling but also the whole picture of host-virus

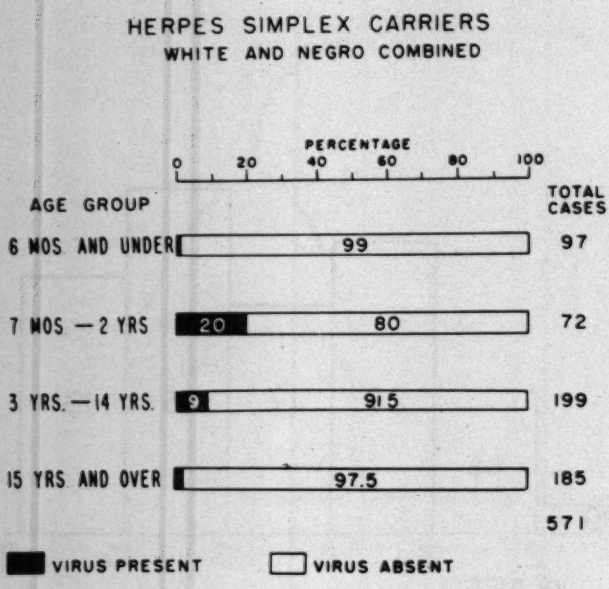

Fig. 3 (Rake). Results of a study of 553 subjects with neutralizing herpes-simplex serologic antibodies. From these carriers the virus could be isolated in varying numbers, depending upon age. It is of interest that 20 percent of the children, aged from seven months to two years, had virus in their mouths, for this is the age range of the highest incidence of inapparent (subclinical) infections. (From Buddingh et al., 1953.)

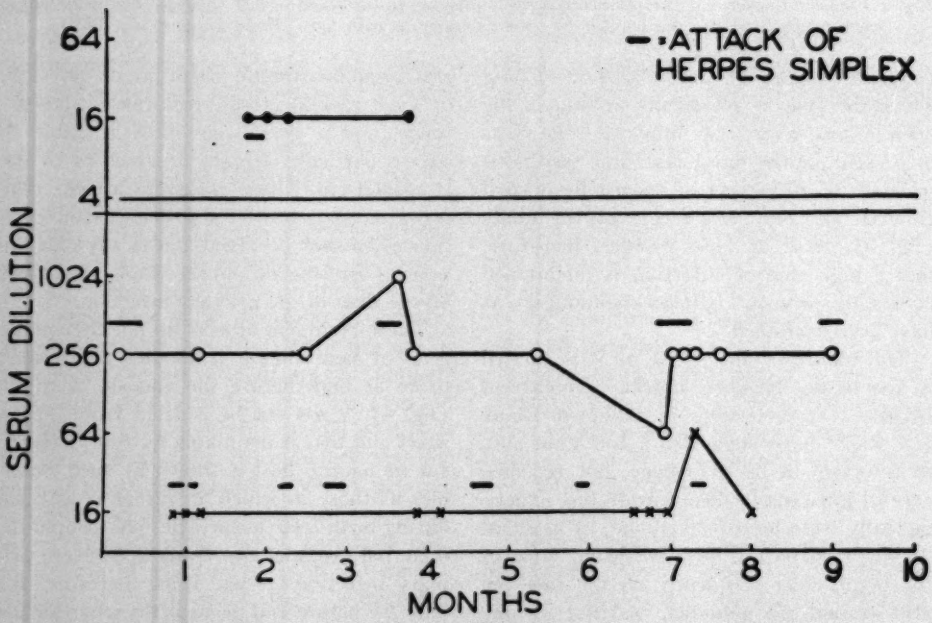

Fig. 4 (Rake). Neutralizing antibody in three adults with recurrent attacks of herpes simplex. The antibody level is unchanged, within experimental error, before, during, or after each attack. (From Scott et al., 1953.)

relationship. If it were not for the fact that the recurrent lesions manifest themselves on the body surface, it is doubtful if the attacks would or indeed could usually be recognized. Under these circumstances one is led to wonder if other viral infections without such superficial manifestations may not behave in manner identical to herpes simplex but escape our recognition.

This story of herpetic infection in man may now be related etiologically to disease of the eye. The eye may be affected during the course of the primary infection when that is overt. It has been pointed out such infection may occur during the act of birth and in such cases the lesions in or around the eye are only incidental in the more general and severe infection. More frequently, and therefore of greater consequence, is the appearance of the primary infection in the form of an acute keratoconjunctivitis. In this condition, from which herpes virus may regularly be isolated, the cornea becomes hazy with superficial ulcerations which eventually heal without scarring, the lids are closed, edematous, and markedly inflamed, and there is a membranopurulent exudate. Usually only one eye is involved. There are herpetic vesicles on the skin around the affected eye. The attack, which lasts from 10 to 20 days, is accompanied by fever.

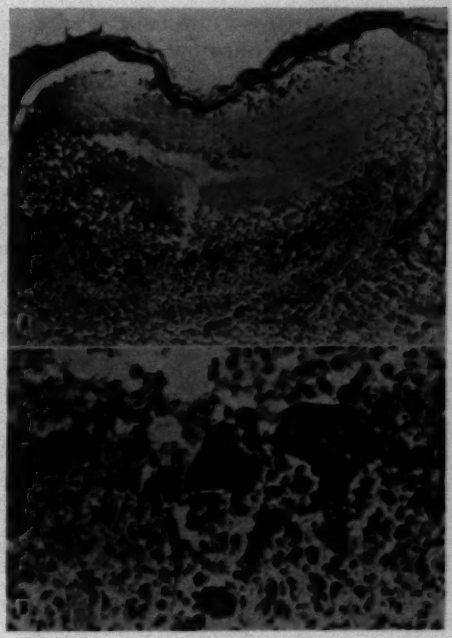

Fig. 5 (Rake). Biopsy of a herpes-simplex vesicle from a Negro. (A) Low-power view (×80), showing the epithelial changes and a relatively clear vesicle. (B) High-power view (×450), showing the multinucleate giant cells forming in the lowermost part of the epidermis. Inclusions can be seen within many of the nuclei of the giant epithelial cells, and melanin is present in their cytoplasm. (From Blank and Rake, 1955.)

Recurrent secondary attacks of herpes may assume any of several manifestations in the eye. Commonest are a dendritic keratitis or a keratoconjunctivitis. Also seen are punctate or marginal keratitis and dendritic corneal ulcers which may progress to produce disciform keratitis. Perhaps due to the introduction of treatment with the corticosteroid hormones, progression to actual hypopyon ulcer is now seen on occasion.[7] In these cases there are usually associated herpetic vesicles on the eyelids and the palpebral conjunctivas. As already stated, the whole attack is of less severity and shorter duration than the primary infection, but the danger exists that such damage, if repeated often, may result in permanent scarring. Despite this serious prognosis, we know of only one possible method which may prevent such recurrent attacks once the pattern for their appearance has been established; that is the removal of the affected cornea and the ongrafting of a new one.[8]

Herpes virus may be isolated from the above recurrent lesions and helps to establish the diagnosis. In one type of case, that of relapsing iritis, the lesion is deep-seated and does not follow the pattern set out above. This iritis may sometimes be hemorrhagic and severe.

Attempts have been made to implicate the virus of herpes simplex etiologically in other diseases of the eye, including epidemic keratoconjunctivitis. It must be emphasized that

this is extremely unwise in the absence of repeated and incontrovertible evidence. Mention has already been made of the ubiquity of the herpetic virus. It has been isolated repeatedly from normal tissues and secretions and from diseased material under conditions that made it clear, at the time or in retrospect, that it was not etiologically involved, but only a chance contaminant. Its isolation, therefore, from a single case of a given eye infection by itself signifies little if anything. Only repeated isolation of the virus, a procedure which is not difficult, or the consistent demonstration in smear or section of the typical appearance of multinucleated giant cells (fig. 5) which Kimura and colleagues have shown to occur as typically in corneal as in skin scrapings, can justify the application of an etiologic association to the finding. This, which is a postulate in the establishment of any viral etiology, is paramount with the virus of herpes simplex.

745 Fifth Avenue (22).

References

1. Buddingh, G. J., Schrum, D. L., Lanier, J. C., and Guidry, D. J.: Pediatrics. **11**:595, 1953.
2. Blank, H., and Rake, G.: Viral and Rickettsial Diseases of the Skin, Eye, and Mucous Membrane of Man. Boston, Little, 1955.
3. Burnett, F. M.: Virus as Organism. Cambridge, Harvard, 1945.
4. Nagler, F. P. O.: Australian J. Exper. Biol. & M.Sc., **24**:103, 1946.
5. Rake, G., and McLimans, W. F.: Unpublished data.
6. Scott, T. F. M., Corriel, L. L., Blank, H., and Burgoon, C. F.: J. Pediat., **41**:835, 1952.
7. Thygeson, P.: Am. J. Ophth., **36**:269 1953.
8. Sezer, N.: Personal communication.
9. Kimura, A., Thygeson, P., and Hogan, M. J.: Exhibit at A.M.A. Convention, Chicago, June, 1956.

LABORATORY DIAGNOSIS OF HERPETIC INFECTIONS OF THE EYE*

Edwin H. Lennette, M.D., and Alwine van Allen, A.B.
Berkeley, California

Of the many virus infections to which man is subject, infection with the herpes simplex virus is probably one of the most common. This statement is based not only on the widespread prevalence, and the frequency of occurrence, of "fever blisters" or "cold sores," but also on the fact that more than 90 percent of the population over 15 years of age possesses neutralizing antibodies to the herpes simplex virus,[1] evidence of previous infection with this agent.

The initial, or primary, infection with the virus of herpes simplex usually occurs in early childhood. Infants born with antibody passively acquired from the mother lose the antibody in approximately six months[1,2] (unpublished observations). The proportion of children with antibodies in the age group six months to two years is low (period of high susceptibility) and then rises, that is, passive immunity wanes, disappears, and subsequently is replaced by an active immunity based on actual infection with the virus[1,3] (unpublished observations). Although primary infections may occur at any age, the majority, according to Blank and Rake,[4] apparently occur during the first five years of life. Probably less than one percent of the primary infections take a clinically overt form.[4]

Primary infection may take any one of several forms clinically recognized at present; these are: herpes simplex (accidental in-

* From the Viral and Rickettsial Disease Laboratory, California State Department of Health.

oculation, through close contact with the virus, into areas of skin whose continuity has been interrupted, either microscopically or grossly), visceral herpes simplex, acute infectious gingivostomatitis, acute herpetic vulvovaginitis, Kaposi's varicelliform eruption, acute infection of the central nervous system, and keratoconjunctivitis. The primary infection, whether frank or subclinical, gives rise to a latent infection which probably persists throughout life. Activation of the latent virus by any of a variety of stimuli produces clinically manifest evidence of the activity of the virus. Recurrent infections may affect the eye, giving rise to a keratitis or keratoconjunctivitis. According to Thygeson,[5] herpetic keratitis, or keratoconjunctivitis, is the most important specific keratitis encountered in the United States.

When present, vesicles on the eyelids and palpebral conjunctivas point to the herpetic basis of the keratoconjunctivitis. In the absence of such clues, the infectious etiology of the condition can be ascertained with certainty only by means of laboratory studies, and, even in the presence of such leads as the appearance of circumorbital vesicles, laboratory studies are desirable to resolve definitively the infectious etiology of the condition.

This paper will be concerned with the procedures involved in the laboratory diagnosis of herpes simplex virus infections of the eye. Dr. Jawetz, Dr. Beale, and perhaps others, will discuss the APC (adnoidal-pharyngeal-conjunctival) viruses or, as they have more recently come to be called, the adenoviruses,[6] and their role in the production of ophthalmic disease. I shall, however, have occasion to refer to the adenoviruses since laboratory diagnostic procedures must take into account the possible involvement of any one of several agents which may give rise to the same clinical picture, in this case keratoconjunctivitis.

Type of diagnostic procedures

In general, the methods available for the laboratory diagnosis of viral diseases may be divided into three broad categories, namely, microscopic examinations, serologic tests, and virus isolation procedures. The usefulness of each of these methods in the diagnosis of herpetic infections of the eye will be mentioned briefly.

Microscopic examinations

These include the examination of fixed and stained tissues for the presence of pathologic or pathognomonic changes, as well as the examination of imprint preparations or smears from tissues, fluids, exudates, and so on, for the presence of inclusion bodies and abnormal cells. The usefulness, on the whole, of microscopic methods is rather sharply restricted as their value in establishing an etiologic diagnosis is limited to a few diseases. With respect to ophthalmology, smears are often made from swabs or scrapings of the conjunctival or corneal surfaces. While the small eosinophilic intranuclear inclusion bodies (Lipschütz bodies) characteristic of herpetic infection of epithelial cells may be present in such smears, the method is too undependable to use as a single diagnostic criterion.

Tzanck and co-workers,[7] and more recently Blank et al.,[8] have observed that pathognomonic "virus type" multinucleate giant epithelial cells are present in smears prepared from the vesicles of herpes simplex, herpes zoster, and varicella, but not in other vesicular diseases of the skin. We are not aware, however, of any reports that these large multinucleated epithelial cells are demonstrable in smears of conjunctival or corneal tissue in herpes simplex virus infections of the eye. In any event, confirmation of the microscopic findings, whether they be positive or negative, should be obtained by other means, either through serologic tests or isolation of the virus, or both.

Serologic tests

The so-called neutralization test is generally employed for the determination of anti-

body levels in serum. The amount of antibody in a patient's serum is ascertained by the extent to which the serum is able to neutralize, or inactivate, the virus, as revealed by inoculation of serum-virus mixtures into suitable test animals, such as the mouse or the embryonated egg. Recently, development of more potent and refined antigens[9] has made feasible a highly specific complement fixation test. Both procedures will be discussed later as to their usefulness and their limitations.

Virus isolation

Isolation of the virus is without question the procedure of choice in the laboratory diagnosis of herpetic infections of the eye. Recovery of the virus represents a significant finding, but negative results do not necessarily exclude the virus as being etiologically responsible for the ocular infection.[10]

Collection of materials for laboratory examination

Collection of material for microscopic examination

At present, smears are of little, if any, value for the diagnosis of herpes simplex infections of the eye. However, because of the information they may provide with respect to the possible presence of other infections, they may, on occasion, be found of some use. In making films or smears, clean slides should be used. The swabbings or scrapings should be spread out as thinly as possible and allowed to dry. It is important that the preparations contain tissue elements in order that the presence of inclusion bodies, pathognomonic cells, or other types of cells will not be missed. Suitable methods for staining are described by Scott in the book listed under reference 10.

Collection of blood specimens for serologic tests

Since assays of serum antibody are often done in living cells (animals, embryonated eggs, or tissue cultures), it is obvious that blood specimens must be taken under aseptic precautions and handled with aseptic techniques and that the bacteriologic sterility of the serum during processing and during storage must be preserved. From 15 to 20 ml. of blood should be taken with a sterile, dry syringe to avoid hemolysis. No anticoagulants or preservatives should be used for reasons which will be mentioned shortly. Whole blood should never be frozen, since freezing will result in total hemolysis of the specimen, thereby rendering it unfit for in vitro serologic tests and possibly making it highly toxic for animals and tissue cultures.

Anticoagulants should not be used in the collection of blood specimens as they may render the serum anticomplementary. Similarly, some preservatives may make the serum anticomplementary, and, in addition, when the serum is also used for neutralization tests, the preservative may have a deleterious effect on the virus. Important details in the collection and handling of blood specimens are given in another publication,[10] to which the interested reader is referred.

Collection of specimens for virus isolation

Proper collection and handling of the specimen is highly important to the successful recovery not only of herpes simplex virus, but of any other viral agents which might be present. The material for laboratory examination should be collected as early as possible in the acute stage of the disease since viruses in general tend to disappear relatively rapidly after the onset of clinical manifestations of infection. Since the material (swabs or scrapings) is to be inoculated into animals or tissue cultures, it should be taken with sterile precautions in order to avoid bacterial contamination, or to add further contamination if micro-organisms are already present.

A dry swab may be used, but is is generally preferable to moisten it with a suitable laboratory solution or diluent (see further below). The swab should be firmly applied

to the affected areas and then placed in a sterile tube containing up to 0.5 ml. of the diluent. Physiologic salt solution, because of its deleterious effect on the herpes virus, should not be used if any other solution, such as nutrient broth, buffered gelatin-saline, skimmed milk, or physiologic saline containing 10 percent of inactivated normal rabbit serum, is available. Because of the importance which tissue culture methods are assuming in the laboratory diagnosis of viral infections, the use of a tissue culture solution such as Hanks' or Earle's balanced salt solution, or a tissue culture medium such as medium 199, is highly desirable; these solutions, containing material leached from the swab, can serve as an inoculum, not only for tissue cultures, but also for animals and embryonated eggs. These solutions also have the added merit of containing several antibiotics, which thus exert a bacteriostatic or bacteriocidal effect.

Unless the material is immediately to be inoculated into suitable laboratory hosts, it should be stored in the frozen state. Storage at dry-ice temperatures is preferable if the necessary facilities are available, since the viability of viruses is best maintained for prolonged periods under these temperature conditions. Lacking such facilities, the material may be stored in a deep freeze unit or in the freezing compartment of a refrigerator. Material stored under these conditions, however, should be tested as soon as possible as the temperature (approximately −15°C.) is too high for the prolonged survival of many viruses.

Laboratory diagnosis

Serologic tests

A serologic diagnosis of a herpetic infection is based upon the appearance or the increase in titer of antibodies in the serum during the course of the illness. To demonstrate the appearance of antibody, or a rise in titer, at least two, and sometimes more, specimens of blood taken during various stages of the illness are required. The first specimen of blood should be taken as early as possible after the onset of the illness, and it should be emphasized that this first specimen cannot be taken too early. The second specimen should be taken two to four weeks after the first. The two specimens are then examined concurrently in the same test; the examinations are conducted by either the neutralization test or the complement fixation test, or both.

Neutralization test. Neutralization tests can be conducted either in mice or in embryonated eggs. We prefer to use mice as this method is less laborious, and we consider the results obtained to be more clear cut, consistent, and reproducible than those obtained in the embryonated egg.

The neutralization test as performed in this laboratory, not only for the herpes simplex virus but for other viruses which are neurotropic for the mouse, is as follows:

A 20-percent suspension (that is, a 2×10^{-1} dilution of the virus) is prepared by grinding infected mouse brain in a mortar and adding, by increments during the course of the grinding, the requisite amount of diluent. A diluent which is widely employed is nutrient broth containing 10 percent of normal rabbit serum which has been inactivated by heating at 56°C. for 20 to 30 minutes. In this laboratory, however, skimmed milk is used. The resultant suspension is centrifuged horizontally for 30 minutes at 3,000 rpm in a refrigerated centrifuge, and the supernatant fluid is carefully removed. This constitutes the stock virus which may be dispensed in the appropriate amounts in small glass ampules which are then flame-sealed and stored on dry ice until required for use.

All sera to be tested are inactivated at 56°C. for 30 minutes. Each serum under examination (for example, paired serum specimens from a patient) is dispensed in 0.15-ml. amounts into a series of tubes. The same procedure is followed with the control sera, which are generally a known normal rabbit serum and a herpes-virus hyperimmune rabbit serum. Serial tenfold dilutions of the

stock virus are prepared in 10-percent normal rabbit serum broth and 0.15 ml. of each of the virus dilutions is added to the serum tubes. This gives a series of serum-virus mixtures in which the serum is undiluted and the final virus concentration is 1 by 10^{-1}, 1 by 10^{-2}, and 1 by 10^{-3}, and so on up to 1 by 10^{-6} or 1 by 10^{-7}.

The mixtures are well shaken and then injected into mice. If baby mice of one to three days of age are used, they are inoculated intraperitoneally with 0.22 ml. amounts, and no incubation of the mixture is required. If adult mice of 28 or more days of age are used, the serum-virus mixture may be incubated for two hours at 37°C. and then injected intracerebrally in 0.03-ml. amounts.

Each serum-virus mixture is inoculated into a group of six or more mice. The animals are observed daily over a period of three weeks, and the presence of symptoms and the occurrence of deaths is recorded. The 50-percent mortality end-point titers (LD_{50} titers) are calculated according to the Reed-Muench formula or the Kürber formula; examples illustrating the computation of end-points by these two methods are given in a recent article by one of us.[10]

The LD_{50} titers are expressed in logarithmic terms. The antibody content of the serum indicated by the LD_{50} titer of the virus in the presence of that serum as compared with the LD_{50} titer of the virus in the presence of a normal, nonantibody-containing serum. Thus, in the case of paired serum specimens examined from a given patient, if we substract the LD_{50} titer of the virus in the presence of the convalescent-phase serum from the LD_{50} titer of the virus in the presence of the acute-phase serum, we obtain a logarithmic number which is referred to as the neutralization index. Generally, the neutralization index is expressed as an arithmetic number, a number which thus indicates the number of LD_{50} of the virus that a convalescent-phase serum can neutralize as compared to the acute-phase serum. This can be illustrated as follows:

Log of LD_{50} titer of virus in presence of convalescent-phase serum — log of LD_{50} titer of virus in presence of acute-phase serum = log of neutralization index. Antilog of neutralization index = arithmetic neutralization index.

Neutralization indices are usually interpreted as follows:

Neutralization index of 1 to 9 = negative. Neutralization index of 10 to 49 = inconclusive. Neutralization index of 50 or more = positive.

In ovo neutralization tests are used to only a limited extent in diagnostic work. The end-point of in ovo neutralization tests with herpes simplex virus is based on the ability of the virus to produce plaques, or pocklike lesions, on the chorioallantoic membrane of the developing chick embryo. The degree of neutralization which occurs is based on the quantitative reduction in the number of virus particles capable of producing lesions, which is indicated by the number of plaques produced. The procedure is time-consuming and open to a number of errors[11] and reading and interpretation of the findings require considerable experience. Determination of the infective titer of the virus alone is apt to yield variable results; estimation of infectivity in the presence of serum gives rise to an additional error which stems from the lack of proportionality between virus dilutions and plaque counts.[12] The reader interested in conducting in ovo neutralization tests with herpes simplex virus is referred to the papers of Burnet and Lush,[13] Scott et al.,[14] and Jawetz and Coleman.[12]

Complement fixation test. In recent years much work has been done on the development of a suitable complement fixation test for the diagnosis of herpes simplex virus infections (see literature review in [9].) A recent paper from this laboratory[9] describes a simple procedure for the preparation of herpetic antigens derived from infected embryonated eggs, as well as a procedure for performance of the complement fixation test itself. The test is highly specific (unpublished data), is sufficiently sensitive to demonstrate rises in complement-fixing antibody when rises in neutralizing antibody are also dem-

onstrable, and has been used for several years in our laboratory for diagnostic work. The literature of virology shows that viral complement fixation tests are performed in a diversity of ways, the variations in technique being referable in part to difficulties engendered by impure and insensitive antigens and in part to personal preferences for methodology. Whatever the technique employed, a diagnostically significant result, that is, a positive finding, is represented by a fourfold or greater rise in antibody titer between the acute-phase and convalescent-phase blood specimens.

Comment

Some comment is required apropos the findings obtained by serologic methods.

During the course of the primary infection, neutralizing antibodies appear and increase in titer during the first week or two of the infection. Presumably as a result of the latent infection which is established consequent to this first invasion of the body by the virus, neutralizing antibody remains at a very high level. Recurrent attacks of herpetic infection are not associated with changes in the antibody level before, during, or after these episodes. Consequently, determination of neutralizing antibody levels in the paired sera of a patient who is suspected to have a herpetic infection provides diagnostically significant information only in the case of primary infections, not in recurrent herpes.

Much the same situation appears to obtain with respect to the complement-fixing antibody. In our experience, rises in complement-fixing antibody titer have appeared only in the case of primary infections. The significance of complement-fixing antibody demonstrable during recurrent episodes of herpes simplex infection is uncertain. By analogy with other viral diseases, complement-fixing antibody to the herpes simplex virus should be of relatively short persistence. It is impossible to state, from the information currently available, whether the complement-fixing antibody persists over long periods of time or whether antibody production on a recall basis is so swift that maximal levels have already been attained by the time the first blood specimen is obtained from the patient. Obviously, studies aimed at a correlation of the findings of the complement fixation and neutralization tests are highly desirable.

Virus isolation procedures

Material collected as described elsewhere above can be tested for the presence of herpes simplex virus by inoculation into a number of laboratory hosts, namely, rabbits, embryonated eggs, mice, or tissue cultures.

Rabbits, once very widely employed, are much less commonly used than heretofore. Inoculation may be done by either the corneal or the intracerebral routes.

Corneal inoculation is done as follows:

Under suitable anesthesia, the eyeball is protruded by firm pressure against the lower lid. The cornea is scarified as thoroughly as possible by a cross-hatch of vertical and horizontal strokes with the point of a sharp scalpel or other instrument. Both eyes should be scarified, and penetration beyond the epithelial layers should be avoided. A swab containing the infected material is rubbed firmly over each scarified cornea. The animals should be watched daily for the development of a keratoconjunctivitis. If the test is negative, only a slight injection of the cornea or the conjunctivas may be present for 24 to 72 hours after the inoculation. If the inoculum contains herpes simplex virus, a keratoconjunctivitis may develop; if it does, it may appear at any time within one to seven days after the inoculation.

Rabbits may also be inoculated intracerebrally with eye swabbings emulsified in a suitable medium. The inoculum consists of 0.25 ml. injected into either hemisphere. Infection with the herpes virus may be manifested in one of several ways:

a. The virus may give rise only to a fever of several days' duration and the animal

will recover and will be immune to challenge inoculation with known herpes simplex virus.

b. The animal may develop a fever which persists for several days and is followed by sudden death without any other symptoms of infection.

c. The animal may develop signs of central nervous system involvement, that is, an encephalitis.

There are several objections to the use of rabbits for the diagnosis of herpetic infections. First of all, herpes simplex virus transferred directly from the patient to the rabbit cornea does not always induce a keratitis in this species. Secondly, distinction cannot always be made between a keratitis induced by the herpes virus from that produced by vaccine virus, or perhaps other viruses, and subpassages or other ancillary identification tests are required.

Embryonated eggs may also be used for the isolation of the herpes simplex virus. Usually, the chorioallantoic route of inoculation is used. By means of a sharp probe, a small hole is made through the shell and shell membrane of 10- to 13-day-old embryonated eggs. A small hole is similarly made over the air sac, and suction is applied by means of a rubber bulb. Withdrawal of the air from the air sac causes the chorioallantois to drop and thus provide a wide flat area of membrane on which the inoculum can be placed. Care should be taken to spread the inoculum (0.05-0.1 ml., or more if desired) as evenly as possible over the membrane. Alternatively, one of the "window" or "shell-flap" techniques may be used, although these are more cumbersome. After incubation for 48 to 72 hours at 35°C. to 36°C., the chorioallantoic membrane is examined for the presence of edematous thickening or plaques, or both. Several passages may be required before typical plaques develop. Difficulty may be encountered in distinguishing pathologic changes induced by the virus from nonspecific effects produced by trauma, certain types of diluents, and so forth. In the hands of experienced workers, however, the technique is suitable for diagnostic purposes.

In our opinion, albino Swiss mice offer many advantages over either the rabbit or the embryonated egg. While adult animals may be used, they are not always suitable for primary isolation of herpes simplex virus because of a certain amount of resistance to infection which develops with increasing age of the animal. In our laboratory, therefore, we employ baby mice of one to three days of age and inoculate 12 animals (two litters of six each) by the combined intracerebral-intraperitoneal route using 0.01 ml. intracerebrally and 0.03 ml. intraperitoneally.

The animals develop weakness, tremor, inco-ordination, and paralysis, and become prostrate and die. Herpetic infection of the baby mouse is frequently associated with the appearance of a purplish tinge to the viscera, quite evident through the thin abdominal wall. The brains from moribund or dead animals are removed, emulsified in normal rabbit serum broth, and the virus is identified by means of neutralization tests in baby mice using known hyperimmune herpes simplex sera.

Recently, the feasibility of using tissue cultures for the isolation of herpes simplex virus has been described by several groups of workers.[15-18] The cellular component has consisted of rabbit kidney, rabbit corneal epithelium, human kidney, or human carcinoma cells (strain HeLa). From information available at this time, we believe that HeLa cell cultures provide the system of choice for the isolation of agents from cases of keratoconjunctivitis. The use of HeLa cell cultures affords an opportunity not only to recover the herpes simplex virus, should this agent be etiologically involved, but also the adenoviruses which have recently been causally implicated. The appearance of type A intranuclear inclusion bodies and the cytopathic changes occurring in HeLa cell cultures infected with herpes simplex virus are

well described by Scherer and Syverton[15] and by Doane, Rhodes, and Ormsby.[16] The cytopathic effects of the adenoviruses on HeLa cells are described by Dr. Jawetz and Dr. Beale elsewhere in this symposium.

The cytopathic effects produced in HeLa cells by the herpes simplex virus differ, and are distinguishable from those produced by the APC, or adenovirus, group. Definitive identification of the herpes simplex virus can be made in monolayer cell cultures using predetermined amounts of the unknown agent, usually 100 TCD_{50}, and several dilutions of a specific hyperimmune serum. The adenovirus group is represented by a multiplicity of immunologic types, and the specific type involved in a given patient or particular outbreak can be determined by this method using type-specific rabbit immune sera. Typing can be greatly expedited by a simple and rapid colorimetric method which will be published shortly from this laboratory.

Summary and Comments

Microscopic examination of smears from the eye is of little, if any, use in the diagnosis of herpetic infections, but may be of use in ruling in or excluding other conditions. Similarly, serologic tests, either neutralization tests or complement fixation tests, are of no value in the diagnosis of recurrent episodes of herpetic infection, although they are highly useful in determining the occurrence of primary herpetic infections.

The diagnostic method of choice for herpetic infections of the eye is isolation of the virus. The difficulties associated with the use of rabbits and embryonated eggs for this purpose are discussed, and, while both hosts can be used, the mouse and the tissue culture systems are considered superior.

The HeLa cell culture system possesses the advantage that it will not only detect the presence of herpes simplex virus, but also of the adenoviruses, including type 8, which has recently been incriminated as a causal agent of keratoconjunctivitis.

California State Department of Health, Berkeley (4).

References

1. Buddingh, G. J., Schrum, D. I., Lanier, J. C., and Guidry, D. J.: Studies of the natural history of herpes simplex infections. Pediatrics, **11**:595, 1953.

2. Scott, T. F. M., Coriell, L. L., Blank, H., and Burgoon, C. F.: Some comments on herpetic infection in children, with special emphasis on unusual clinical manifestations. J. Pediat., **41**:835, 1952.

3. Scott, T. F. M., et al.: Cited by Blank and Rake.[4]

4. Blank, H., and Rake, C.: Viral and Rickettsial Diseases of the Skin, Eye, and Mucous Membranes of Man. Boston, Little, 1955.

5. Thygeson, P.: Herpes corneae. (Editorial.) Am. J. Ophth., **36**:269, 1953.

6. Enders, J. F., Bell, J. A., Dingle, J. H., Francis, T., Jr., Hilleman, M. R., Huebner, R. J., and Payne, A. M.-M.: Adenoviruses: Group name proposed for new respiratory tract viruses. Science, **124**:119, 1956.

7. Tzanck, A.: See Blank et al.[8]

8. Blank, H., Burgoon, C. F., Baldridge, G.D., McCarthy, P. L., and Urbach, F.: Cytologic smears in diagnosis of herpes simplex, herpes zoster, and varicella. J.A.M.A., **146**:1410, 1951.

9. Sosa-Martinez, J., and Lennette, E. H.: Studies on a complement fixation test for herpes simplex. J. Bact., **70**:205, 1955.

10. Lennette, E. H.: General principles underlying laboratory diagnosis of viral and rickettsial infections. Chapter in Diagnostic Procedures for Virus and Rickettsial Diseases. New York, Am. Pub. Health Assoc., 1956, ed. 2.

11. Burnet, F. M., and Faris, D. D.: The teachinque of quantitative chorioallantoic virus titration. J. Bact., **44**:241, 1942.

12. Jawetz, E., and Coleman, V. R.: Studies on herpes simplex virus: III. The neutralization of egg-adapted herpes virus by human sera *in ovo*. J. Immunol., **68**:645, 1952.

13. Burnet, F. M., and Lush, D.: Herpes simplex: Studies on antibody content of human sera. Lancet, **1**:629, 1939.

14. Scott, T. F. M., Coriell, L. L., and Blank, H.: The diagnosis of infection with the virus of herpes

simplex. In Horsfall, F. L., Jr.: Diagnosis of Viral and Rickettsial Diseases. New York, 1949. (See also chapter by Scott.[39]

15. Scherer, W. F. and Syverton J. T.: The viral range in vitro of a malignant human epithelial cell (strain HeLa, Gey): I. Multiplication of herpes simplex, pseudorabies, and vaccinia viruses. Am. J. Path., **30**:1057, 1954.

16. Doane, F., Rhodes, A. J., and Ormsby, H. L.: Tissue culture techniques in the study of herpetic infections of the eye. Am. J. Ophth., **40**:189 (Nov. Pt. II) 1955.

17. Beale, A. J., and Hair, H. C.: Rapid diagnosis of the herpetic variety of Kaposi's varicelliform eruption by tissue culture methods. Canad. M. A. J., **74**:443, 1956.

18. Sosa-Martinez, J., Gutierrez-Villegas, L., and Sosa, R. M.: Propagation of herpes simplex virus in tissue cultures of rabbit kidney. J. Bact., **70**:391, 1955.

STUDIES ON HERPES SIMPLEX

VIII. The significance of isolating herpes-simplex virus from the eye

L. Hanna, M.A., E. Jawetz, M.D., and V. R. Coleman, B.A.
San Francisco, California

The virus of herpes simplex is known to be widely distributed in most human populations. From serologic studies it is evident that from 50 to 90 percent of all adults have been infected with the agent. It is assumed that the large majority carry the virus in some latent form and that it can be brought into activity by a suitable trigger mechanism. In view of the widespread distribution of this agent, sometimes referred to as its "ubiquitous presence," the questions arise: what significance should be attributed to the isolation of herpes simplex virus from humans? Is the virus liberated constantly or intermittently for long periods in persons who have acquired the infection? Or can it be isolated only when specific active lesions of herpes are present? Can it be found on the normal appearing eye, or mouth, of carriers? If so, it might be found frequently in the course of virologic studies and confuse the assignment of an etiologic role to this, or other viruses.

In the course of our studies on the etiology of various forms of external eye disease we isolated during 1955-56 20 strains of herpes simplex virus. It is the purpose of this paper to examine the methods by which these viruses were isolated and to determine the significance of herpes virus in the clinical picture. An attempt will also be made to answer some of the questions asked above with particular reference to the eye.

Materials and Methods

Patients

Specimens were obtained from 283 patients whose diagnoses are listed in Table 1. They fall into two broad groups:

I. Patients with clinical herpes. II. Patients with nonherpetic clinical features, or normals.

Among the cases grouped as "keratitis" in II (table 1) there were superficial or epithelial keratitis of unknown origin, superficial punctate keratitis, keratitis sicca, herpes zoster, nonherpetic bullous keratopathy, and marginal ulceration. "Keratoconjunctivitis" in II included five cases of epidemic keratoconjunctivitis, in addition to trachoma, and all other forms. "Conjunctivitis" included vernal conjunctivitis, blepharoconjunctivitis, inclusion blenorrhea, acute and chronic catarrhal conjunctivitis, and pharyngoconjunctival fever. From the last named entity several types of adenovirus (APC virus) were isolated which have been considered in other publications.[1,2] The "miscellaneous" group in

TABLE 1

DISTRIBUTION OF ISOLATION OF HERPES-SIMPLEX VIRUS FROM PATIENTS ACCORDING TO CLINICAL DIAGNOSES*

Diagnosis	Virus Isolated	Attempts	% Isolation
I. Patients with clinical herpes			
A. Superficial keratitis			
Dendritic	14	45	
Geographic	1	10	
Punctate	0	0	
Diffuse	0	2	
Total	15	57	26.3%
B. Stromal			
Disciform	2	26	
Disciform with ulceration	0	2	
Hypopyon ulcer	0	3	
Kerato-uveitis	0	1	
Total	2	32	6.2%
II. Patients without clinical herpes			
Keratitis	1	77	
Keratoconjuctivitis	2	24	
Conjunctivitis	0	67	
Miscellaneous eye disease	0	15	
Normal	0	11	
Total	3	194	1.5%

* Clinical diagnoses established by Dr. S. J. Kimura and Dr. P. Thygeson.

II was made up of cases of ocular pemphigus, Parinaud's conjunctivitis, xeroderma pigmentosum, epithelial dystrophy, and so forth.

SPECIMENS

Patients were examined, and specimens obtained, in the eye clinic of the University of California School of Medicine or the Proctor Foundation. We are indebted to many ophthalmologists who referred patients for study.

Specimens were obtained either by scraping cornea and conjunctiva with a sterile platinum spatula or curette, or by washing the conjunctiva and cornea repeatedly with sterile tissue culture medium. Whenever possible the specimen was inoculated directly onto embryonated eggs or into HeLa cell tissue cultures. If that was not possible, the specimens were quick frozen in 0.4 ml. tissue culture maintenance medium (10 percent chick serum in mixture 199 containing antibiotics) and stored in sterile screw-capped tubes in a mechanical refrigerator at −20°C. for varying periods, up to five weeks.

VIRUS ISOLATION AND IDENTIFICATION

Specimens were inoculated onto the chorioallantoic membrane of embryonated eggs, or into HeLa cell tissue cultures.* A very few specimens were also inoculated onto rabbit's cornea. Most specimens were insufficient to permit inoculation of more than one host tissue, so that no adequate comparative data on the reliability of different methods for such virus isolation from the eye became available. It is known, however,

* From the Departments of Microbiology and Ophthalmology and the Proctor Foundation for Research in Ophthalmology, University of California Medical Center. Supported by grants from the National Institutes of Health (B 604), Burroughs, Wellcome and Company, and the Committee on Research, University of California School of Medicine. Part of a comprehensive study on keratoconjunctivitis carried out by a group, including: L. Hanna, E. Jawetz, S. J. Kimura, A. Nicholas, P. Thygeson.

that the three methods are roughly comparable in sensitivity to herpes simplex viruses.[3]

The chorioallantoic membrane of 12-day-old embryonated eggs was dropped by the false air sac technique. One tenth or 0.2 ml. were placed on the dropped membrane, the window sealed with Scotch tape, and the eggs returned for additional incubation. After 48 or 72 hours the chorioallantoic membranes were harvested and examined for typical lesions. If the lesions were few or atypical one or two egg passages were performed. Finally the isolated virus was identified by neutralization with specific rabbit antiserum to herpes simplex.

HeLa cell tissue cultures* were washed twice with Hanks* solution to remove all traces of the human serum contained in growth medium. The specimen in a volume of 0.1 or 0.2 ml. was placed on the cell sheet and the maintenance medium (10 percent chick serum in mixture 199) containing penicillin 500 units/ml., streptomycin 500 μg/ml., 10 μg/ml. tetracycline and 10 μg/ml. rimocidin, added. The tubes were incubated in a stationary position at 36°C. for as long as the integrity of the cell sheet permitted, usually 14 to 21 days. Tubes were inspected daily for evidence of cytopathogenic effects. If degeneration occurred the virus was established by additional passage in tissue culture and identified by neutralization with specific antiserum.

Neutralizing antibodies to herpes simplex virus were determined on acute and convalescent serum specimens obtained from certain patients by the usual techniques of neutralization tests in mice, or eggs, as described earlier.[4]

Results

Twenty strains of virus identified as herpes simplex were isolated from 283 patients (table 1). Seventeen strains originated in patients clinically diagnosed as herpes simplex and only three strains in the 194 patients with other clinical diagnoses. Thus the recovery of herpes simplex virus from nonherpetic disease occurred in only 1.5 percent of patients. One of these strains came from a keratitis of unknown etiology, the two others from patients with clinical epidemic keratoconjunctivitis.

The keratitis of unknown etiology occurred in a 41-year-old seaman seen seven days after the onset of red, watering eyes. Six weeks earlier the patient had been hospitalized for bacterial pneumonia and had suffered a penicillin reaction. The referring ophthalmologist considered the eye disorder also to be a pencillin reaction. There was no history of any clinical herpes. Examination showed bilateral epithelial lesions, palpable preauricular nodes, and reduced corneal sensitivity. Cytologic examination showed mainly polymorphonuclear leukocytes and occasional monocytes. No follow-up on the patient was feasible, but the possibility exists that a dendritic figure may have appeared subsequently, so that the patient suffered from clinical herpes.

The two isolations of herpes viruses from patients with epidemic keratoconjunctivitis have been reported previously.[5] Other investigators have had similar experiences.[6,7] Yet it seems quite certain that these viruses are not the etiologic agents of epidemic keratoconjunctivitis. Both of our patients had a significant rise in antibodies to adenovirus type 8 during the illness and there is ample evidence of the consistent association of the latter virus with clinical epidemic keratoconjunctivitis.[8] The possible significance of herpes simplex virus in epidemic keratoconjunctivitis is discussed later.

Most strains of herpes virus were isolated from patients with a definite clinical picture suggesting this etiology. Among 57 individuals in various stages of superficial herpetic keratitis 15, or 26.3 percent, yielded

* HeLa cell tissue cultures were obtained from the Carver Foundation, Tuskegee Institute, Alabama. All tissue culture media were obtained from Microbiological Associates.

the etiologic virus. Regrettably many of these individuals were seen rather late in their disease so that the chances of virus isolation were small. In most instances specimens were obtained on one occasion only. Multiple attempts did not seem to increase the percentage of isolations. Among 32 patients with stromal herpetic lesions the virus was recovered from only two or 6.3 percent. This is in keeping with the experience of Braley[9] who found it possible only rarely to isolate a virus from these deep lesions.

To the ophthalmologist who desires to confirm his clinical impression of a herpetic keratitis by isolation of the etiologic virus the method of obtaining a specimen is of considerable importance. Should he collect superficial exudate, cell debris, and desquamated cells, and extracellular, free virus by washing; or should he scrape off infected, superficial cells which have not yet broken down? We attempted to find the answer in the following comparison:

In 45 cases of dendritic keratitis in various stages of development we attempted isolation of virus. From 17 of these, washings only were obtained—and only a single virus recovered (6.0 percent). From 24 cases scrapings were obtained and 12 viruses isolated (50 percent). In four other patients both washings and scrapings were pooled and virus was recovered once. Thus it seems evident that scrapings from dendritic keratitis offer a far better chance—10 times better—of recovering herpes simplex virus than washings. The hypothesis that scraping might release local antibody and thus mask virus is evidently not based on fact.

In the course of our work it was not feasible to compare the efficacy of the two methods of virus isolation, chorioallantoic membrane and HeLa cell tissue culture. Too few specimens were examined adequately by both methods to permit any conclusion. Of 24 specimens from dendritic keratitis tested only in HeLa cells, four yielded a virus; of 17 specimens tested only on chorioallantoic membrane, five yielded a virus; and from 12 specimens examined by both methods five viruses were recovered. It is our impression that either method may be satisfactory, but that chorioallantoic membrane inoculation may be slightly more sensitive. This is supported by the experimental results with egg-adapted herpes virus shown in Table 2.

TABLE 2

RELATIVE SUSCEPTIBILITY OF CHORIOALLANTOIC MEMBRANE (12 DAY) AND HELA CELL CULTURES TO EGG-ADAPTED HERPES SIMPLEX (HF)

	No. Pocks on Chorioallantoic Membrane	Cytopathogenic Effect on HeLa
1	80	+
2	45	+
3	15	−
4	12	+
5	10	+
6	5	−
7	5	−
8	3	−

It appears that about 10 pock-producing infective units are required to produce cytopathogenic effects in the HeLa cultures we employed. It has also been our experience that certain freshly isolated herpes viruses stored for several months at −20°C. failed to produce degeneration in HeLa tubes while resulting in readily transmissible lesions on chorioallantoic membrane which could be neutralized by specific antiserum. No isolations resulted from the few inoculations of rabbit cornea which we performed.

DISCUSSION

In our past work with herpetic lesions of the skin we were able to isolate the virus from at least 80 percent of early (that is, unbroken) herpetic vesicles. The recovery of herpes virus from only 26.3 percent of dendritic keratitis in comparison appears to be unsatisfactory. However, it must be considered that a skin vesicle contains a very large number of infected epithelial cells which all remain confined to the area, whereas in the cornea loose cells, debris, and free

virus tend to be removed promptly by lacrimal flow and lid action. Thus it is necessary to detach infected cells from the corneal epithelium by scraping in order to obtain a satisfactory specimen. Even then the amount of infective virus secured in the limited number of abraded cells is evidently too small to be recovered regularly. Another important feature probably lies in the unfortunate lapse of time between onset of acute keratitis and our obtaining the specimen.

In spite of the obvious limitation of our data they permit an answer to some of the questions asked. Herpes virus cannot be isolated readily from eyes that appear normal, nor from a variety of forms of conjunctivitis and keratitis of unknown etiology. Thus no support was found in this work for the claims of Busacca in 1925[10] and Gruter[11] who recovered herpes virus from normal eyes. Our findings are in agreement with the work of Kilbourne and Horsfall[12] who failed to isolate herpes virus from one patient, using throat or mouth washings, except at those times when active lesions were present. Buddingh et al.,[13] on the other hand, isolated herpes virus from seven percent of 571 individuals without evident mouth lesions.

It is evident that lesions on the cornea are far more difficult to miss than mouth lesions. Our results suggest that a good deal of confidence can be placed in the diagnostic value of isolating herpes simplex from the eye. If the virus is isolated, it represents the etiologic agent of the lesion in the vast majority of instances.

False positives occurred in only 1.5 percent of the cases. The most important of these were patients with epidemic keratoconjunctivitis. Several other investigators[6,7] have also isolated herpes or herpeslike viruses from that disease.

A partial explanation might lie in the possible diagnostic confusion between epidemic keratoconjunctivitis and acute herpetic keratoconjunctivitis. However, that is probably not the entire explanation. Our epidemic keratoconjunctivitis cases had a high and constant titer of antibodies to herpes simplex and a significant rise in antibodies to adenovirus type 8 during their illness. Thus they definitely did not suffer from primary herpetic infection during their clinical epidemic keratoconjunctivitis. It is conceivable that epidemic keratoconjunctivitis has a specific tendency of activating latent herpes simplex in the eye, or even that herpes virus in some way contributes to the clinical picture, although it is not the primary etiologic agent. Future work may elucidate these points.

For the time being it is our experience that:

Recovery of herpes simplex virus from the eye occurs only in lesions of herpetic etiology with the exception of some cases of epidemic keratoconjunctivitis. It is present far more regularly (and in higher concentrations) in keratitis involving predominantly superficial epithelium than in stromal involvement. However, like Braley,[9] we have recovered the virus from occasional disciform lesions.

The biggest problem, the nature of herpetic latency in the eye, remains to be studied. By analogy to known systems, the virus could either reside intracellularly as a noninfective "provirus," which can be transmitted by the cell to its daughters in the process of division, and requires a trigger for activation; or it could be present in fully infective form, kept latent by antibody which masks its presence unless the balance is disturbed by some trigger. Experimental analysis, as yet, has provided no answer.

Summary

1. In a series of 283 individuals, specimens from the eye yielded only 20 strains of herpes simplex; 15 of these were derived from cases of dendritic keratitis, and two from disciform lesions. Among 194 persons who lacked the clinical stigmas of herpetic keratitis, only three yielded herpes simplex.

Two of the latter suffered from epidemic keratoconjunctivitis.

2. Specimens from cornea and conjunctiva obtained by scraping and curettement are far more likely to yield herpes simplex than simple washings.

University of California Medical Center (22).

References

1. Jawetz, E., Hanna, L., Kimura, S. J., and Thygeson, P.: A new type of APC virus from follicular conjunctivitis. Am. J. Ophth., **41**:231-234, 1956.

2. ———: Clinical diseases of adults associated with sporadic infections by APC viruses. Arch. Int. Med., **98**:71-79, 1956.

3. Coriell, L. L., Blank, H., and Scott, T. F.: Isolation of herpes simplex virus on the chorioallantoic membrane. J. Lab. & Clin. Med., **34**:402-408, 1949.

4. Jawetz, E., and Coleman, V. R.: Studies on herpes simplex virus: III. The neutralization of egg-adapted herpes virus by human sera *in ovo*. J. Immunol., **68**:645-653, 1952.

5. Jawetz, E., Kimura, S., Hanna, L., Coleman, V. R., Thygeson, P., and Nicholas, A.: Studies on the etiology of epidemic keratoconjunctivitis. Am. J. Ophth., **40**:200-211 (Nov. Pt. II) 1955.

6. Maumenee, A. E., Hayes, G. S., and Hartman, T. L.: Isolation and identification of the causative agent in epidemic keratoconjunctivitis (superficial punctate keratitis) and herpetic keratoconjunctivitis. Am. J. Ophth., **28**:823-839, 1945.

7. Moritsch, H.: Uebertragungsversuche mit dem Virus der Keratoconjunktivitis. Zentralbl. Bakt., **160**:168-170, 1953.

8. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., and Kimura, S.: Antibodies to APC virus type 8 in epidemic keratoconjunctivitis. Proc. Soc. Exper. Biol. & Med., **92**:91-95, 1956.

9. Braley, A. E.: Experimental herpes simplex. Am. J. Ophth., **35**:1737-1747, 1942.

10. Busacca, A.: Latenter Augenthalt von Herpes virus in Konjunktivalsack und posttraumatischer Herpes corneae. Arch. f. Augenh., **95**:253-260, 1925.

11. Gruter, W.: Das Herpesvirus, seine atiologische und klinische Bedeutung. Munch. Med. Wchnschr., **71**:1058-1060, 1924.

12. Kilbourne, E. D., and Horsfall, F. L.: Primary herpes simplex virus infection of the adult. Arch. Int. Med., **88**:495-502, 1951.

13. Buddingh, G. J., Schrum, D. I., Lanier, J. C., and Guidry, D. J.: Studies on the natural history of herpes simplex infections. Pediatrics, **11**:595-610, 1953.

THE EFFECT OF CORTICOSTEROIDS ON EXPERIMENTAL HERPES SIMPLEX KERATOCONJUNCTIVITIS IN THE RABBIT*

S. J. Kimura, M.D., and M. Okumoto, M.A.
San Francisco, California

Much confusion surrounds the use of corticosteroids in herpes simplex infection of the eye. In previous reports we have emphasized the ineffectiveness of cortisone in treating clinical dendritic ulcers, and the danger of using the more potent hydrocortisone.[1] The purpose of the present study was to determine the effect of the corticosteroids, especially hydrocortisone and prednisolone, on experimental herpes simplex keratitis in the rabbit.

Materials and Methods

Animals. Albino and colored rabbits, weighing approximately four lb., were supplied by a commercial breeder.

Virus. The experimental virus was the mouse-brain-adapted PH strain (O strain[2]) of herpes simplex virus in its 12th to 16th passage. Its infectivity titer for mice by the

* From the Department of Ophthalmology and the Francis I. Proctor Foundation for Research in Ophthalmology, University of California School of Medicine.

intracerebral route was from $10^{-3.7}$ to $10^{-5.6}$ per 0.03 ml.

Virus inoculation. Two different inoculation procedures were followed:

1. During the first part of the study a direct corneal scratch method was used: two horizontal and two vertical scratches were made on the cornea with a No. 21 hypodermic needle which had been dipped in a 2×10^{-1} viral suspension.

2. During the latter half of the project the virus was injected into Tenon's space so that the amount of inoculum could be measured.

Corticosteroid hormones. Hydrocortisone* in both the acetate and the free alcohol forms, and prednisolone acetate† were injected into the subconjunctival space, or into Tenon's space, in a volume of 0.2 ml., on the day of the virus inoculation and every third day thereafter.

Experimental herpes simplex keratoconjunctivitis

When herpes simplex virus was scratched on the rabbit cornea, a dendritic ulcer usually developed on the scratch in from 24 to 48 hours. The time of onset of the clinical disease seemed to vary according to the concentration of the inoculum. There was usually an associated mild conjunctivitis with slight mucous discharge. The eye was only slightly inflamed and often only close examination revealed the presence of keratitis. The keratitis and conjunctivitis healed completely in from 10 to 14 days.

Injection of the virus into Tenon's space was found to be far superior to the scratch method for the purpose of studying a developing experimental herpes simplex keratoconjunctivitis. A known amount of virus could be introduced into the potential space surrounding the greater part of the globe. The keratoconjunctivitis took longer to develop (72 to 96 hours) but the keratitis, in the form of multiple small dendritic ulcers distributed over the whole cornea, was highly reproducible. It healed, like the lesions induced by scratch inoculation, in from 10 to 14 days.

The corticosteriod effect

Experimental conditions were manipulated in various ways in an effort to explore the dynamics of the system by which the corticosteroids affect herpetic infection of the eye. The variables included the following:

1. Route of viral inoculation: corneal scratch versus injection into Tenon's space.
2. Amount of virus inoculated.
3. Type of corticosteroid: hydrocortisone versus prednisolone.
4. Type of hydrocortisone: acetate versus free alcohol.
5. Dosage of the corticosteroid.

The results of varying these factors in two series of experiments may be described as follows:

1. Following inoculation of the cornea by the scratch method: By this method the amount of the inoculum could not be controlled. A 2×10^{-1} dilution was therefore used to insure a corneal "take." Treatment with hydrocortisone acetate, hydrocortisone free alcohol, and prednisolone acetate, in a total dosage of from 20 to 50 mg. (10 mg. every third day), resulted in the progression of the herpetic keratoconjunctivitis to encephalitis and death in a high percentage of cases. Increasing the dosage of hydrocortisone free alcohol fourfold to 40 mg. every third day did not alter the results in any way. Of the 19 rabbits treated, 11 died between the ninth and 24th days after inoculation, presumably from encephalitis. They were found dead in their cages, and virus recovery was not attempted. Five of the rabbits were killed when signs of encephalitis were recognized, and in four of these herpes simplex virus was recovered from the brain. Only three rabbits survived and in one of

* Furnished by Dr. Alpert of Merck and Company.

† Furnished by Dr. Pifer of the Schering Corporation.

the three the eye perforated; the remaining two showed marked corneal scarring.

Of 19 control rabbits, all recovered within 14 days with scarring that was visible only with magnification.

2. Following inoculation of the virus into Tenon's space: Four rabbits inoculated with 0.03 ml. of 10^{-2} or 10^{-3} dilutions of the virus suspension were treated with prednisolone acetate. All four developed equally severe keratoconjunctivitis (fig. 1), regardless of the dilution of the inoculum. In two the infection progressed to encephalitis and the rabbits died. The other two survived but were killed on the 21st and 23rd days after inoculation when their corneas ruptured.

Six control rabbits were inoculated with 0.03 ml. of 10^{-1}, 10^{-2}, or 10^{-3} dilution of the suspension and left untreated. In all six, regardless of the dilution of the inoculum, the characteristically mild keratoconjunctivitis (fig. 2) developed and healed in from 11 to 14 days.

Discussion

Experimental herpes simplex infection of the eye can be made worse by treatment with corticosteroid hormones. The mechanism by which this occurs is not clear. The high incidence of death due to encephalitis in the treated animals suggests an enhancement of the generalized spread of the virus by the corticosteroids, and/or increased multiplication of the virus. Viral titration studies are under way in an attempt to test the latter possibility.

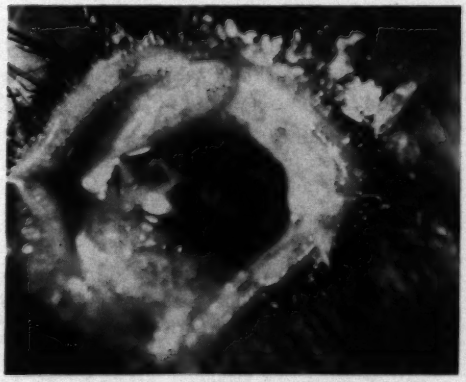

Fig. 1 (Kimura and Okumoto). Rabbit eye (treated with predniosolone acetate) eight days after injection of Tenon's space with 0.03 ml. PH M16 strain of herpes simplex virus.

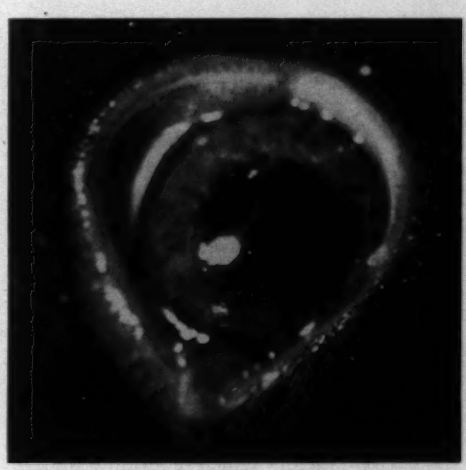

Fig. 2 (Kimura and Okumoto). Rabbit eye (untreated control) eight days after injection of Tenon's space with 0.03 ml. PH M16 strain of herpes simplex virus.

Conclusions

Under the conditions of the experiments, treatment of experimental herpes simplex keratitis with hydrocortisone produced encephalitis and death in a high percentage of cases. The rabbits that survived had a more severe keratoconjunctivitis and their corneas frequently ruptured.

In a small series of rabbits in which the keratoconjunctivitis was produced by injecting herpes simplex virus into Tenon's space, and in which the induced disease was treated with the newer synthetic steroid, prednisolone acetate, death from encephalitis occurred in only 50 percent, although the severity of the corneal lesions differed in no way from the severity of the lesions treated with hydrocortisone acetate or hydrocortisone free alcohol.

University of California
Medical Center (22).

REFERENCES

1. Thygeson, P., Hogan, M. J., and Kimura, S. J.: Cortisone and hydrocortisone in ocular infections. Tr. Am. Ophth. Soc., Jan.-Feb., 1953, pp. 64-85.
2. Jawetz, E., Coleman, V. R., and Merrill, E. R.: Studies on herpes simplex virus. VII. Immunological comparison of strains of herpes simplex. J. Immunol., **75**:28-34, 1955.

EPIDEMIOLOGY OF HERPETIC INFECTIONS*

T. F. McNAIR SCOTT, M.D.
Philadelphia, Pennsylvania

Maxcy defines[1] epidemiology as "the field of science dealing with the relationship of the various factors which determine the frequencies and distributions of an infectious process, a disease, or a physiologic state in a human community." He then proceeds to consider these factors under various headings which I believe will be useful ones for us to follow in this discussion of the epidemiology of herpetic infections.

HOST-PARASITE RELATIONS

Since "infectious disease is a manifestation of parasitism," as first clearly enunciated by Theobald Smith[2] just over 20 years ago, an evaluation of an infectious disease demands a study of both parasite and host.

It must be emphasized that the herpes virus is a very successful parasite in that it causes a high ratio of subclinical to clinical infections, so that the infected hosts remain carriers in the community without great disability to themselves. The host is man alone: no other animal gets a natural infection with this virus although among monkeys and hogs there are natural infections with parasites of the herpes group, for example, B. virus and Pseudorabies virus respectively.

The relationship between the herpes virus and its host is a more intimate one than that of many parasites since it is so highly adapted to its environment that it is able to live in the host's body for many years in the form of a latent virus causing little or no clinical evidence of disease. Historically, this peculiar relationship led to considerable confusion about herpetic infection.

The parasite had been isolated as early as 1912 from herpetic keratoconjunctivitis and later (1919) from the blisters of herpes simplex, and had been studied quite extensively in the laboratory from 1920 to 1930. In the latter year Andrews and Carmichael[3] observed that 74 percent of 53 normal adults had circulating antibodies against this virus and that seven individuals subject to frequently recurring attacks of herpes labiales all had circulating antibodies.

It had also been a matter of clinical observation that attacks of herpes seemed to be provoked by some nonspecific stimulus, such as fever, and did not spread from person to person. These findings were quite contrary to the usual concept of an infectious disease, and such a prominent virologist as Doerr[4] in 1938 suggested "that herpes is not an infectious agent which is maintained by a chain of infection but that it is endogenously generated in the human organism."

In that year a combination of clinical acumen and further laboratory study by Dodd and her co-workers[5] in this country and Burnet and Williams[6] in Australia, respectively,

---

* Director of Research, Children's Hospital of Philadelphia and Research Professor of Pediatrics, School of Medicine, University of Pennsylvania.

clarified the situation by demonstrating that the herpes virus caused a stomatis in young infants who had no circulating antibodies and that on recovery they developed circulating antibodies.

In other words the herpes virus did act as an external parasite causing an antibody reaction in the usual way when it invaded a susceptible host. However, instead of the parasite disappearing from the host's body with recovery from the acute disease, as is the case with most parasites, the virus of herpes remains in the tissues of the host and, at intervals, causes local injury to that tissue when the balance between host and parasite is disturbed by some change in the physiology of the host. Table 1 illustrates our present concept of the host-parasite relationships.

With this broad concept in mind it is now possible to discuss in more detail other epidemiologic facets of this infection.

Host reaction

This may be severe leading to clinical manifestations, or mild and subclinical.

A. Clinical

Recognition of the clinical picture is essential for a clear understanding of the epidemiology of any disease. In herpetic infections this may be difficult because these manifestations are quite protean and the full extent of them may not as yet have been described. However, up to the present it is possible to recognize lesions of the skin, in the form of herpes simplex, eczema herpeticum, traumatic herpes; of the mucous membranes as acute gingivostomatis, vulvovaginitis, herpes progenitalis; of the eye as keratoconjunctivitis; of the central nervous system as encephalitis; as a generalized illness both in adults, resembling grippe, infectious mononucleosis, or smallpox, and in newborn infants in whom a fatal involvement of the brain, lungs, adrenals, and liver may occur.

These various forms are outlined in Table 2. All these have been classified as manifestations of primary herpetic infection on the basis of laboratory studies to be referred to later. Recurrences occur on the skin, the genitalia, and the eye.

B. Subclinical

The reaction may be so mild that it is only recognizable by the application of laboratory methods. These fall essentially into two categories: (1) Isolation of the virus, and (2) measurement of antibodies. There are three recognized antibodies measured at present: (a) Neutralizing, (b) complement fixing, and (c) skin test.

It is a general principle that the recognition of the etiology of an infectious disease depends on the isolation of the agent and/or the appearance during convalescence of circulating antibodies against a given agent

TABLE 1

Host-parasite relationship of herpes simplex

Susceptible host − virus = Primary Infection (No circ. antibodies)	
Primary Infection =	a) Manifest disease = local lesion + systemic illness
	b) Subclinical infection = No visible disease
Recovery	= Carrier state (circulating antibodies)
Carrier State	= a) Recurrent disease = local lesion usually without systemic illness
	= b) Latent infection = no visible illness
Carrier State → Virus → Infection of susceptible host	

TABLE 2

Diseases caused by virus of herpes simplex

Skin	Mucocutaneous Junction
Herpes simplex	Herpes labialis
Eczema herpeticum	Herpes progenitalis
Traumatic herpes	Vulvo vaginitis
Mucous Membranes	**Eye**
Acute gingivistomatitis	Conjunctivitis
Recurrent stomatitis???	Keratoconjunctivitis
Central Nervous System	
Meningo-encephalitis	
Generalized Infection	
Newborn and adults	

which were not there at the onset of the illness. In the primary infection with herpes virus this principle can be fully applied. However, in the recurrent disease the picture is somewhat different and since this has considerable bearing on epidemiology the two aspects will be considered separately.

Primary infection

The virus is readily isolated from the area involved; of more interest is the serologic response. Figure 1 illustrates the rise in neutralizing antibody in 31 children studied at intervals after their acute infections. It can be seen that the titer rose steeply between the fourth and eighth days[7] and then remained at a plateau for the 18 days that they were followed. However, this tendency to plateau at a constant level cannot be extrapolated indefinitely.

Buddingh and his co-workers[8] followed 12 children for several weeks after their acute infection and found a considerable variation in the length of time that antibody levels persisted. In one child the neutralizing antibody level persisted at a high level for 12 weeks while in another it had dropped to low levels by the fourth week (fig. 2).

It has been pointed out by others also that, in children, the neutralizing antibodies may disappear below the level of detectability in time, for example, in one child the titer was high during an active keratitis but was hardly detectable 16 months later,[9] while in another it had disappeared between five and eight months after recovery from eczema herpeticum.[10] In one case such fluctuations appeared to coincide with the appearance of the virus in the saliva without any evidence of clinical disease (fig. 3). It would seem, therefore, that during childhood a single primary infection may not allow the parasite to establish itself but that repeated subclinical infections may have to occur until a balance is achieved and the host maintains a constant level of antibody.

Recurrent infection

Although the virus can also be isolated from the lesions in such cases, the antibody response is different. For the most part it would appear that circulating antibody is present at the time of the attack and does not alter following the attack. This is illustrated by Figure 4 in which neutralizing antibodies were measured in three adults each of whom had recurrent disease although the frequency of the attacks differed. It can be seen that, over a period of four months, there was no significant change in the level of neutralizing antibody in relation to an attack. While this absence of reaction is probably the usual situation it has been shown that some adults react to a recurrent attack by a rise in antibodies either neutralizing, complement fixing, or both.[11]

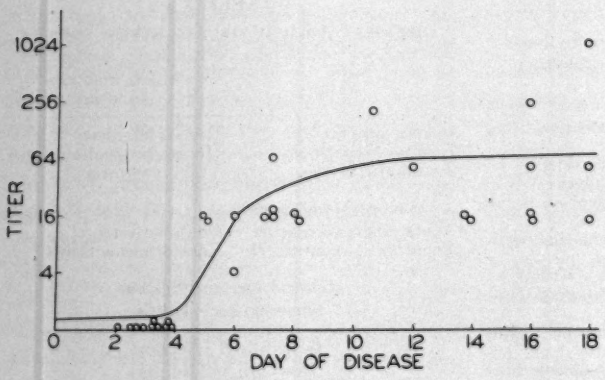

Fig. 1 (Scott). The rise in neutralizing antibody in 31 children studied at intervals after their acute infections. (From Scott, et al.)

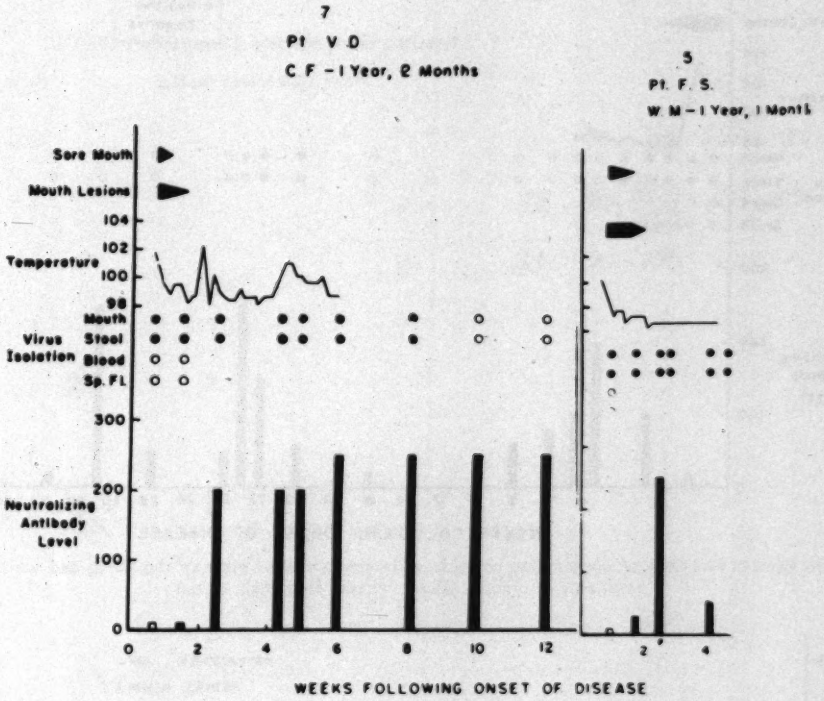

Fig. 2 (Scott). Showing variation in the length of time that antibody levels persist. (From Buddingh, et al.)

Population at risk

These data have significance when another facet of epidemiology is studied, namely the population at risk. As will be illustrated later the incidence of subclinical infection by this parasite is much larger than is the clinical, therefore the absence of a history of a primary illness is not of great value in determining the incidence of susceptibles. This must be done by measuring the presence of antibodies in the population at large.

A. Techniques involved

This theoretically can be done by any one of the three available techniques, neutralization test, complement-fixation test, or skin test. The first appears to be the most sensitive in that Holzel et al.[12] have shown that occasionally a positive neutralization test can be found in the absence of a positive complement-fixation test but never vice versa. It has the disadvantage, however, of expense and of being time consuming.

The complement-fixation test is cheaper and quicker and is being widely used for survey purposes. That it parallels the neutralization test very closely can be seen from the results obtained in our laboratory (fig. 5).

When skin tests are available, they are usually the most advantageous for survey purposes and it was hoped that the herpes skin test might be useful in this regard. However, in some unpublished observations[13] we have found that in the population over 50 years of age there is a decrease in the incidence of positive skin tests despite the presence of complement-fixation and neutralization antibodies. Furthermore, in

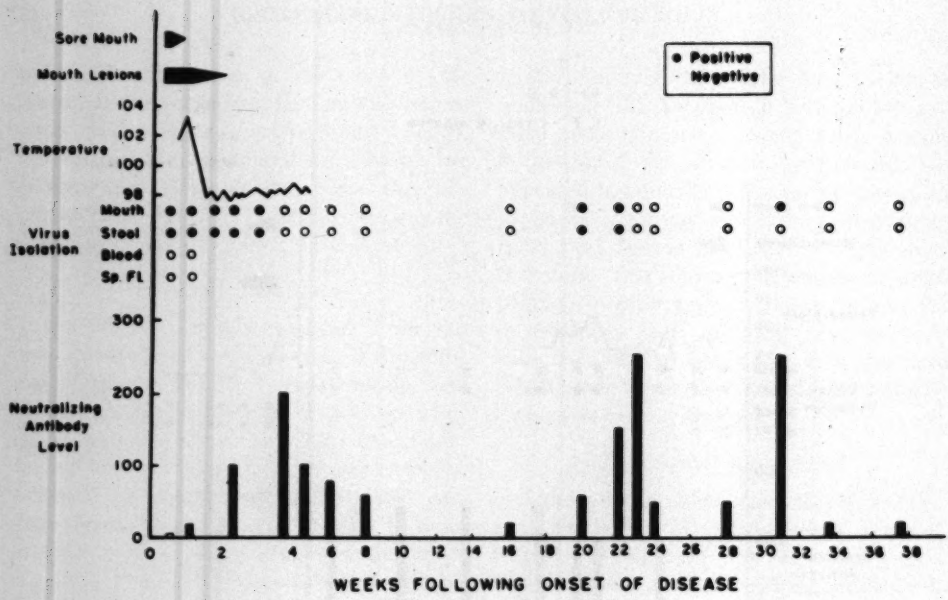

Fig. 3 (Scott). Fluctuations appeared to coincide with appearance of virus in the saliva and without any evidence of clinical disease. (From Buddingh, et al.)

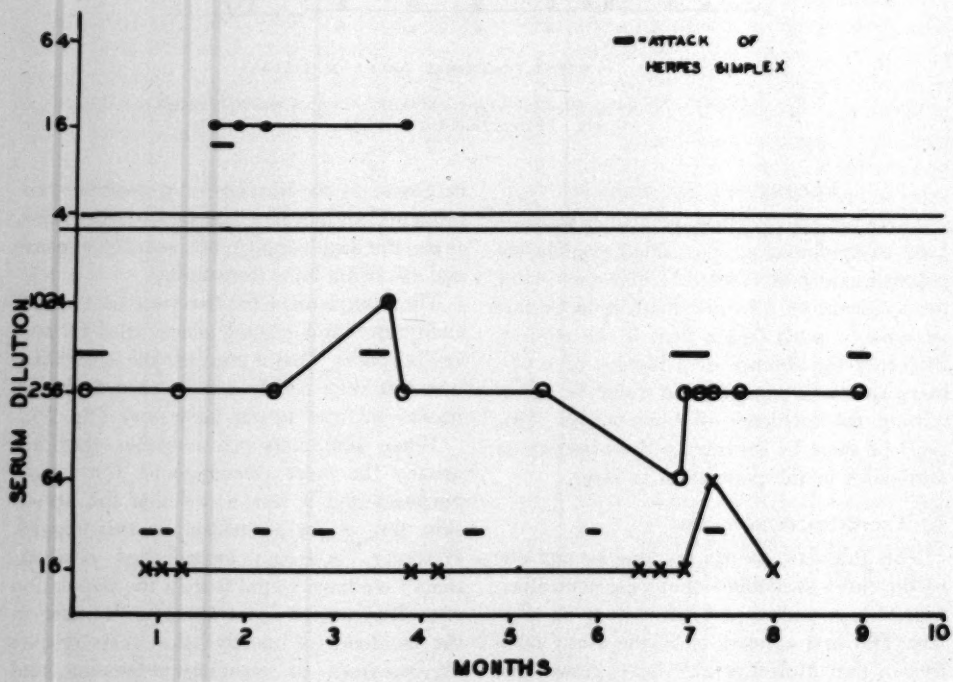

Fig. 4 (Scott). Neutralizing antibody level in recurrent herpes simplex. (From Scott, et al.)

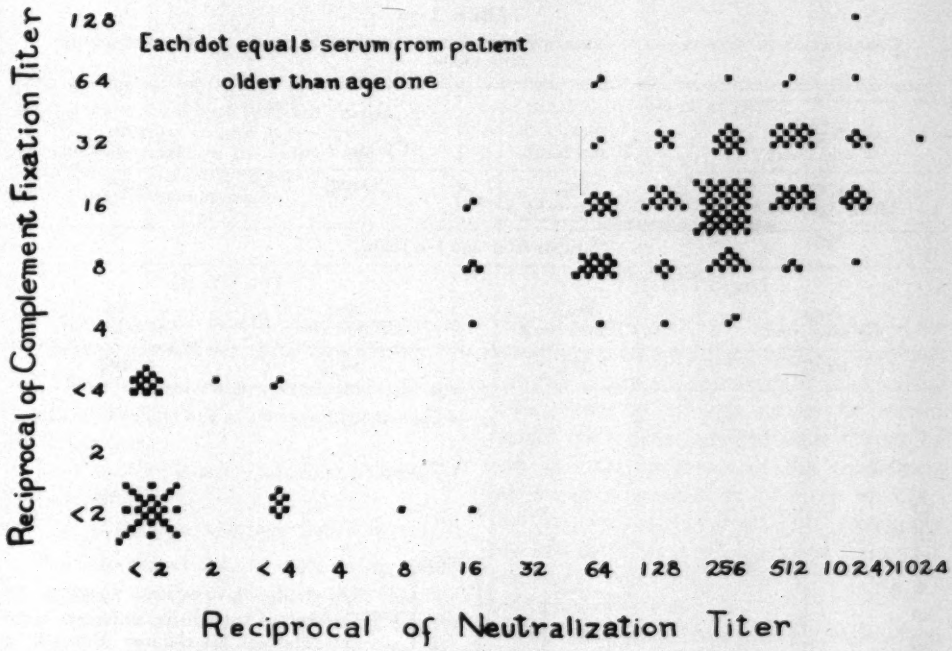

Fig. 5 (Scott). Comparison of complement-fixation and neutralization tests.

infancy, skin tests are notoriously unreliable. In older childhood and young adulthood, however, there is excellent parallelism among all three tests (table 3).

B. Survey results

From the available surveys two sets of facts have emerged:

1. *Socio-economic.* This is an important factor influencing the incidence of herpetic infections. This was apparent in the early 1930 survey of Andrews and Carmichael[3] who reported a lower incidence of persons with antibodies among medical students than among assorted hospital patients but this was not further studied at the time.

Burnet and Lush[14] in Australia before World War II reported 93 percent of 55 persons attending a public hospital and 37 percent of 27 medical students and graduates had circulating antibodies against herpes. In Philadelphia among the lower income groups attending the out-patient clinic of the Children's Hospital of Philadelphia in 1947-48 we found an incidence of 64 percent.[7] The significance of these observations will be discussed later.

2. *Age.* It has been shown that the greatest incidence of susceptibles is between six and 24 months of age,[6,7,15] as illustrated by our study of 131 Philadelphia children (fig. 6). It can be seen that very few children in this age period had antibodies but by the age of five years the incidence of infections had reached that of the adult population of the area.

From Figure 6 it is also possible to deduce that herpes neutralizing antiobdies are transferred through the placenta, since the incidence of infants with circulating antibodies during the early months of life, when passively acquired antibodies could be expected, is the same as that in the adult population.

Figure 7 from Anderson and Hamilton's

TABLE 3

CORRELATION OF SKIN TEST AND SEROLOGY WITH HERPES SIMPLEX IN PATIENTS OVER THE AGE OF NINE YEARS

Neg. S.T. (46)		Pos. S.T. (136)	
Neg. CF and Neut.	Pos. CF and Neut.	Neg. CF and Neut.	Pos. CF and Neut.
42% Mean Age = 16.4	58% Mean Age = 54.5	3%	97%
Patients from age 1–8 years			
Neg. S.T. (11)		Pos. S.T. (4)	
Neg. CF and Neut.	Pos. CF and Neut.	Neg. CF and Neut.	Pos. CF and Neut.
100%	0%	50%	50%

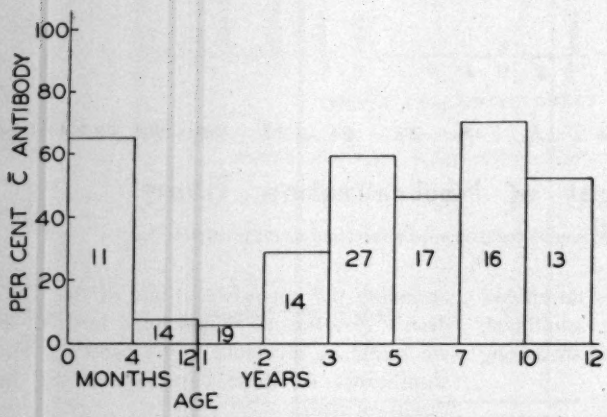

Fig. 6 (Scott). Incidence of herpes neutralizing antibodies in the blood of 131 children. Numbers in each box indicate number of children tested at each age group.

paper[15] illustrates an interesting feature of this infection, namely, that the age of susceptibility and the age of incidence of clinical disease do not correspond. It can be seen that, although in this instance antibodies were absent from seven to 15 months, infections did not appear until 11 months despite what must have been adequate exposure to the virus since, in the orphanage where the studies were undertaken, 100 percent of susceptibles became infected by the time they were 22 months of age.

The loss of detectable antibody by the sixth month suggests that the herpes antibody follows the normal pattern of passively transferred immune gamma globulin since it is in this fraction that the herpes antibodies are carried. However, it is possible that the resistance to the virus between seven to 11 months is due to the persistence of antibody in amounts below the sensitivity of our assay methods. On the other hand, since about 40 percent of the adult population in the Philadelphia area do not have circulating antibodies, it seems strange that the clinical impression is that there are very few cases of stomatitis in clinical practice occurring below the age of one year.

If proof can be obtained that infants of noninfected mothers are also resistant until

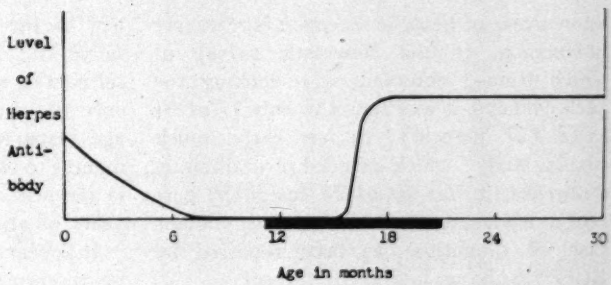

Fig. 7 (Scott). The age of susceptibility and the age of incidence of clinical disease do not correspond. (From Anderson and Hamilton.[35]) Black bar represents period when clinical disease appeared.

over the age of 12 months then there are at least three possibilities to be considered:

1. That the mucous membranes of the mouth at this age are more resistant to infection than later.
2. That there is less virus in the homes of these children.
3. That there is a dosage factor involved.

The importance of the last is suggested by several reports in the literature that infants between six and 12 months of age do suffer from eczema herpeticum. In such instances there is a large area of traumatized skin available for infection so that the dose of virus applied to the child must be many times greater than would be applied to the much smaller area of mucous membrane involved in a stomatitis.

Ratio of subclinical to clinical infections

Discussion of the incidence of infections as determined by serologic survey leads naturally to a presentation of the evidence for the statement made previously that the incidence of subclinical was higher than that of clinical infection. Actually there are very few data available on this point and I only know of two studies.

In one my colleagues and I[7] tried to determine the incidence of herpetic stomatitis among the out-patient population of the Childrens' Hospital of Philadelphia by requesting that we be called to see every case that presented itself during the course of a year. Thirty-eight cases were diagnosed by laboratory methods among 5,016 patients under five years of age seen in the medical clinic. Since by antibody survey, 64 percent should have been infected with herpes by that age the incidence of the commonest clinical manifestation of primary infection was just over one percent. This is certainly too low since we probably missed seeing a few cases and mild cases would not be brought to the clinic.

The other study, which may give too high a figure, since in this case the diagnosis was purely clinical, was undertaken by Spence and his colleagues[16] in Newcastle-on-Tyne in England as part of a study of illness among a thousand families. In the first three years of life among an average population of 950 children they diagnosed 64 cases. The average incidence of infection among the adult population in the city was not determined but the families studied consisted of a cross section of economic strata with the majority falling into a medium economic group. If, then, we estimate that the rate of infection was 60 percent, as it was in our clinic population, we find an incidence of 11 percent. It is probably fair then to assume that at least 90 percent of herpetic infections are subclinical.

Reservoir of infection

It is clear that the virus must be acquired by a susceptible host from another human being carrying the virus. Several studies have indicated that a history of contact with a

known case of herpetic infection is relatively uncommon. In the Newcastle survey in which trained observers were looking for such contacts, it was found in only 17 of 64 cases (27 percent); in our early, much smaller study[17] which included several family outbreaks, it was 10 of 21 cases (50 percent), while a contact history with another case of stomatitis has been reported by Black[18] to be as low as 10 percent.

These data must mean that the virus is carried by persons without clinical manifestations of herpetic infection. The existence of these carriers has been demonstrated and they have been shown to fall into two groups, those that carry the infectious agent for a period immediately following their acute disease even though clinically recovered, and those in whom the virus appears without reference to preceding or accompanying clinical manifestations.

In regard to the former we studied four children recovered from herpetic stomatitis who had no clinical evidence of disease and were able to demonstrate virus in two on the 13th and 14th day, in one on five occasions up to seven weeks, then no virus in the 10th and 20th week but reappearance in the 23rd week. In the fourth patient no virus was found on the 10th day.[17]

Black[19] found virus to be present in the mouth of an experimentally infected child on the 5th, 12th, and 34th day after inoculation, although her mouth had been clinically well for 16 days before the last culture. It was not present on 40th day.

This variability in the persistence of the virus is well illustrated by Buddingh and his co-workers, who found herpes virus in both saliva and stools in seven children who appeared perfectly well from 15 to 42 days after the onset of their illness with an average of 23 days. Figure 3 illustrates the disappearance and reappearance of virus in one of these patients following an acute illness.

Buddingh et al. have also studied the problem of the healthy carrier by testing the saliva of 571 individuals at various ages selected at random and found that the presence of virus in the saliva decreases with age, from 20 percent of children seven months to two years, when primary infection is frequent, to 2.5 percent in those over 15 years of age (fig. 8).

It appears from these data that the greatest reservoir of infection is among children with an inapparent infection. Whether these percentages can be applied to other population groups in other locations is not known and the question deserves further study.

I would like now to refer to some very sparse data we collected in 1941[17] which is suggestive and I think needs further follow-up. We wondered about a reservoir among the parents of our patients and particularly the mother. We investigated 13 parents, four fathers and nine mothers. No virus was found on the single occasion each was tested in the four fathers nor in seven of the nine mothers, all of whom had circulating antibodies. However, in two mothers examined more than once an interesting correlation between presence of virus and menstruation suggested itself.

In one mother no virus was found in her intermenstrual period but was found six days later when the period was expected. In the second, who was examined on three occasions, no virus was found on one occasion 11 days after the onset of menstruation and after cessation of the flow but virus was found on two occasions, once four days after onset, and once at a time which, in retrospect, was the time of a period.

Incubation period

Because of the ubiquitous nature of the virus, the exact length of the incubation period has been difficult to estimate. Among 33 patients studied we calculated that this was three to nine days and might be longer in some cases. Spence and his colleagues[16] in Newcastle found that seven days was the

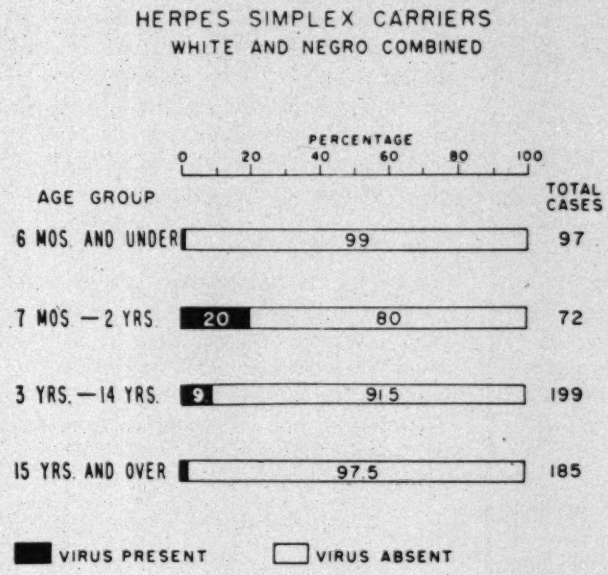

Fig. 8 (Scott). The presence of virus in the saliva decreases with age. (From Buddingh, et al.)

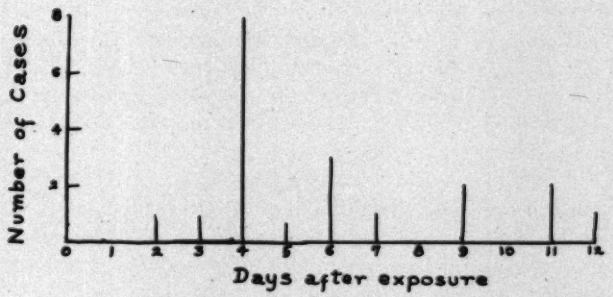

Fig. 9 (Scott). Herpetic stomatitis incubation period in 20 cases. (From data supplied by Dr. Miro Juretić of Split, Jugoslavia, 1955.)

incubation period in the majority of cases.

Dr. Juretić of Yugoslavia[20] has sent me his observations on 23 families involving 56 persons with either stomatitis or herpes simplex. If, from his graphs, the first contact case only is utilized then it is possible to plot the incubation periods of 20 cases. These range from two to 12 days with a peak incidence on the fourth day (fig. 9).

In a child who burned her finger and whose mother had labial herpes the wound began to show evidence of herpetic infection on the third day (Scott et al.[7]). In the child reported by Black[19] fever followed by stomatitis occurred on the third day after she had been experimentally inoculated with the contents of a herpetic vesicle onto scarified gums. It would appear then that herpes infection, despite its variability, tends to have a relatively short incubation period comparable to other infections that affect the skin or mucous membranes.

Epidemics of herpetic infection

Because of the high incidence of subclinical disease there are very few data on epidemics. What there are can be classified as:

1. Family epidemics

In 1941, we described three family epidemics.[17] In one family there were five proven cases and one doubtful case with age ranges from three and one-half to 11 years. In the second, there were three proven cases with age ranges from two and one-half to six and one-half years and, in the third, there were three proven cases, two children 20 months and five years, respectively, and a 36-year-old mother. Table 4 illustrates the first of these families.

In 1944 Chilton[21] described the appearance of stomatitis in six or eight children in a Negro family following the proven diagnosis in the oldest child of 13 years. Juretić[20] has studied 23 families with more than one member affected. In 18 there were two members, in two there were three, in two there were four, and in one there were six.

2. Ward epidemics

Pugh, Dudgeon, and Bodian[22] described an epidemic in the skin ward of the Hospital for Sick Children, Great Ormond Street, London. Case 1 was admitted on September 8th and, on September 9th, was thought clinically to be suffering from Kaposi's varicelliform eruption although this was not confirmed in the laboratory. On September 15th Case 2 developed proven Kaposi's varicelliform eruption and died 10 days later. Between September 18th and 21st two other children with eczema developed Kaposi's varicelliform eruption and two nurses developed vesicular eruptions on their forearms and hands which were proven to be due to herpes.

3. Institutional epidemics

Anderson and Hamilton[15] described the spread of infection in an orphanage in Australia in a study already referred to. They found that every one of 36 susceptible children between the ages of 11 and 22 months developed herpetic infection, 20 with clinical evidence of stomatitis and 16 subclinically.

Mechanism of spread

Two possible mechanisms can be suggested: (1) That the virus is airborne, and (2) that the virus is conveyed by direct contact.

Against the former must be placed the evidence derived from sources in different parts of the world that there is a real difference between socio-economic groups which is certainly not true in the case of airborne infections in general.

The contagious hypothesis is favored by the above findings since with the increased crowding which accompanies the decrease in economic status the infection rate from contagion would increase.

The epidemiology of herpes is very similar to that of poliomyelitis in this regard,

TABLE 4

Family epidemic of herpetic stomatitis
(From Scott and Steigman)

Patient	Age (yr.)	Day Illness	Neutralizing Index		Change in Neutralizing Index
			Acute	Convalescent	
EBr (initial case)	10	7	1,429	700	− 2 fold
RBr	11	5	2	1,429	+714 fold
VBr	8	2	1	769	+769 fold
LBr	7	2	7	2,000	+286 fold
CBr (father)	36	not sick	10,000	1,000	− 10 fold

especially since Buddingh et al. showed that herpes virus could be isolated from the stools as well as the saliva of patients with clinical disease or from carriers.[8] It was not entirely clear as to the origin of the stool virus but the most likely explanation was that it was swallowed in sufficiently large quantities to make it detectable in the rectal swabs. It did not appear in the stools until three or four days after it was detectable in the saliva.

The possibility of actual infection of the alimentary tract below the mouth, must however, be considered. Presumably close physical contact is required for the spread of the virus from either saliva or by the fecal-oral route. Early infancy is a period of life during which close bodily contact is part of everyday life.

The infrequent primary infection of the adult can usually be traced to physical contact also, as from kissing, which leads to an attack of stomatitis, or sexual intercourse during which the male with herpes progenitalis may give rise to a primary vulvovaginitis in the female. In regard to the former Burnet[23] quotes Mercutio's lines from Romeo and Juliet:

> O'er ladies' lips, who straight on kisses dream
> Which oft the angry Mab with blisters plagues
> Because their breaths with sweetmeats tainted are.

It would seem that a trigger mechanism might be involved in determining whether a clinical or subclinical infection might take place. Teething in the infant leads to traumatized gums which might form the point of entrance for the virus. We studied a young adult woman, married for 10 years to a husband who suffered recurrent attacks of herpes labialis who did not get her primary infection until she started to massage her gums vigorously on her dentist's orders.[24] Infection of the skin is almost always due to the inoculation of virus directly on to the traumatized areas, for example, a graze or a burn or weeping eczema.

Recurrent herpes

The epidemiology of recurrent herpes necessitates a consideration of changes in the physiology of the patient. These can be considered under various headings:

1. External environment

Sunburn or irritation from cold winds as forerunners of an attack of labial herpes are common experiences.

2. Internal environment

a. *Fever.* The occurrence of facial herpes in acute febrile states such as pneumonia and meningococcal infection has long been recognized. Even artificially induced fever without infection led to an attack of herpes in 46 percent of 411 patients treated by Carpenter et al.[25]

b. *Menstruation.* The association between attacks of recurrent herpes and the menstrual period is again a common clinical experience.

c. *Nerve injury.* It has been noted for many years that herpes occurred in a high percentage of patients following trigeminal sensory root section. Carton and Kilbourne[26] recently made a study of this phenomenon and found that 16 of 17 patients, who were herpes carriers and were operated upon by trigeminal sensory root section for tic douloureux, developed herpetic vesicles on the side of the operation. Virus was isolated from the vesicles but not from the nerve material removed. From the absence of this reaction in one patient who had absent pain sensation due to a previous operation, the authors speculate that herpes virus is activated by a change in local physiology of the skin subsequent to the interruption of previously functioning nerve impulses.

d. *Emotion.* A recent study by Blank and Brody[27] has re-emphasized the importance of emotion in inducing an attack of recurrent herpes. They point out that the persons with frequent recurrences appear to fall into a

personality pattern, which is characterized by passivity and submissiveness. They were "good, sweet" people who readily accepted psychiatric referral and who were emotionally immature and dependent. On two occasions when the therapist cancelled an appointment, resentment was aroused and an attack of herpes resulted within 24 hours of the cancelled appointment.

Heilig and Hoff[28] were able to produce an attack of herpes in three carriers within 24 to 48 hours of reminding them, under hypnosis, of an unpleasant affective situation, and Schneck[29] reported the case of a soldier who could anticipate an attack after an emotional strain involving feelings of hostility unless he were able to channel these feelings in other ways, that is, by getting drunk. It seems probable that the interest of the physician may be the essential factor in the control of these attacks by such non-specific treatment as X-ray therapy or smallpox vaccination.

Conclusion

There has been no reference to the epidemiology of ocular herpes in this review, since there appeared to be no readily accessible published material on this aspect. Although primary infection of the eye is recognized clinically, I am aware of only one published study, by Gallardo,[30] in which such an infection was actually proven. Thanks to the courtesy of Dr. Leopold, I had the opportunity to review about 150 charts of cases of herpetic ocular infection at the Will's Eye Hospital in Philadelphia. A superficial examination made it clear that a precipitating factor was not spontaneously described by the majority of patients. Among the minority, from whom such a factor was elicited in a routine history, trauma and systemic infection, for example, pneumonia, appeared about equally.

Children's Hospital
of Philadelphia (46).

References

1. Maxcy, K. F.: Epidemiology. Philadelphia, Lippincott, 1952, chap. 7: Viral and rickettsial infections of man. (Ed., T. M. Rivers). Phila, Lippincott, 1952.
2. Smith, T.: Parasitism and Disease. Princeton, N.J., Princeton, 1934.
3. Andrews, E. H., and Carmichael, E. A.: Lancet **1**:857, 1930.
4. Doerr, R.: Herpes febrilis. In Doerr, R., and Hallauer, C.: Handbuch d. Virusforchung. Wien, Springer, 1938, v. 1, p. 41.
5. Dodd, K., Johnston, L. M., and Buddingh, G. J.: J. Pediat., **12**:95, 1938.
6. Burnet, F. M., and Williams, S. W.: M. J. Australia, **1**:637, 1939.
7. Scott, T. F. McN., Coriell, L. L., Blank, H., and Burgoon, C. F.: J. Pediat., **41**:835, 1952.
8. Buddingh, G. J., Schrum, D. I., Lanier, J. C., and Guidry, D. J.: Pediatrics, **11**:595, 1953.
9. Jawetz, E., and Coleman, V. R.: J. Immunol., **68**:655, 1952.
10. Wenner, H. A.: Am. J. Dis. Child., **67**:247, 1944.
11. Dascomb, H. E., Adair, C. V., and Rogers, N.: J. Lab. & Clin. Med., **46**:1, 1955.
12. Holzel, A., Feldman, G. V., Tobin, J. O., and Harper, J.: Acta paediat, **42**:206, 1953.
13. Gray, A., and Scott, T. F. McN.: Unpublished observations.
14. Burnet, F. M., and Lush, D.: Lancet, **1**:629, 1939.
15. Anderson, S. G., and Hamilton, J.: M. J. Australia, **1**:308, 1949.
16. Spence, J., Walton, W. S., Miller, F. J. W., and Court, S. C. M.: A Thousand Families in Newcastle-on-Tyne. London, Oxford, 1954.
17. Scott, T. F. McN., and Steigman, A. J.: J.A.M.A., **117**:999, 1941.
18. Black, W. C.: Am. J. Dis. Child., **56**:126, 1938.
19. ———: J. Pediat., **20**:145, 1942.
20. Juretić, M.: Personal communication, 1955.
21. Chilton, N. W.: Am. J. Orthodontics, **30**:335, 1944.
22. Pugh, R. C. B., Dudgeon, J. A., and Bodian, M. J.: Path. & Bact., **69**:67, 1955.
23. Burnet, F. M.: Virus as Organism, Cambridge, Harvard, 1954.
24. Rogers, A. M., Coriell, L. L., Blank, H., and Scott, T. F. McN.: New England J. Med., **241**:330, 1949.
25. Carpenter, C. M., Boak, R. A., and Warren, S. L.: J. Exper. Med., **71**:155, 1940.

26. Carton, C. A., and Kilbourne, E. D.: New England J. Med., **246**:172, 1952.
27. Blank, H., and Brody, M. W.: Psychosom. Med., **12**:254, 1950.
28. Heilig, R., and Hoff, M.: Med. Klin., **24**:1472, 1928. (Quoted by Dunbar, H. F.: Emotions and Bodily Changes. New York, Columbia, 1938.)
29. Schneck, J. M.: Psychosom. Med., **9**:62, 1947.
30. Gallardo, E.: Arch. Ophth., **30**:217, 1943.

CORNEAL TRANSPLANTATION IN THE TREATMENT OF HERPETIC DISEASE OF THE CORNEA*

MICHAEL J. HOGAN, M.D.
San Francisco, California

I have become impressed with the increased number of patients having chronic corneal ulcers and keratitis occurring spontaneously and as the result of antibiotic and corticosteroid therapy of corneal herpes infections. A number of these lesions have resisted all types of treatment. Some have proved to be examples of secondary infection, at times of fungus origin, superimposed upon the original herpetic disease. They were characterized by chronicity, progressive necrosis, and even perforation. Others proved to be examples of chronic stromal herpes infection of the disciform or ulcerative type, with or without extension to the anterior uveal tract.

Experimental and clinical investigations soon proved to us that herpes infections could be spread by some of the therapeutic measures utilized to control the infection. Over a period of years we had seen and treated many corneal herpes cases and only rarely observed such severe types of corneal inflammation.

The adoption of different methods of treatment failed to result in amelioration of symptoms in most of the advanced cases. Finally, after months of temporizing, and following the advice of other observers, it was decided to perform various types of keratoplasty on some cases, My interest was heightened in this entire problem by the fact that it might be possible through keratoplasty to prevent the constant recurrences observed in many corneal herpes patients. Accordingly, keratoplasties were performed on all patients with the following types of corneal herpes, provided there were no other contraindications, and if the visual acuity in the eye was reduced sufficiently to justify such surgery:

1. Herpetic corneal scars, inactive, but in which there was a strong tendency for recurrence of dendritic keratitis: (a) Superficial, diffuse; (b) disciform stromal scars.
2. Cases of chronic superficial stromal herpes, with ulceration (metaherpetic keratitis).
3. Chronic diffuse postherpetic keratitis with necrosis, ulceration, and vascularization, and with or without perforation.

DISCUSSION OF INDICATIONS

1. THOSE WITH HEALED POSTHERPETIC SCARS

The scarring may be diffuse and superficial in type, or localized in the form of a disciform lesion in the deeper stroma. Such patients may have surgery to prevent recurrences if they occur with such frequency as to be disabling; also to improve vision if the scarring does not clear within a reasonable period after the acute attack. Franceschetti

* From the Francis I. Proctor Foundation for Research in Ophthalmology and the Department of Ophthalmology, University of California School of Medicine.

and Maeder[1] state that such cases are among the most favorable.

2. Chronic superficial ulceration

Those cases with chronic superficial ulcerations which follow a typical dendritic keratitis have been designated as having metaherpetic ulcers, postherpetic disciform ulceration, or as chronic herpetic ulceration of the cornea. Surgery in such cases is performed during the active stages only if healing cannot be accomplished by ordinary methods. In many such patients in our series who were referred for therapy, the disease had persisted for considerable periods and resisted all the usual therapeutic measures.

3. Chronic keratitis, necrosis, vascularization

Those patients with chronic keratitis and necrosis and vascularization, with or without perforation, usually had prolonged topical and oral therapy for postherpetic keratitis, mostly with antibiotics and corticosteroids. Superficial or deep ulceration, scattered areas of necrosis, and progressive vascularization in one or more areas are the outstanding findings. Perforation may or may not have occurred. Evidence was presented recently[2] that a number of these cases resulted from a secondary infection by fungi, and that antibiotic and corticosteroid treatment favored such infections. Several of the cases observed by us had similar findings.

The surgical treatment of such cases is undertaken with the knowledge that the prognosis is poor. However, I consider it to be justified to advise surgical intervention. The results are occasionally gratifying.

Those cases with ulceration which is secondary to bacterial or fungus infections should not be rejected, because in most instances the infection is chronic, and the prognosis without surgery is hopeless. I have not encountered a single instance of early postsurgical intraocular suppurative infection in this type of case.

Contraindications to keratoplasty in herpetic keratitis

The contraindications to such surgery in patients with the various types of herpes affections of the cornea are much the same as for other corneal conditions.

Secondary glaucoma may or may not be in evidence prior to surgery, but should be kept under consideration because of the recurrent or chronic inflammation. In a few of our cases it developed in severe form in the postoperative period, even though the surgery itself was accomplished without difficulty.

A coincident iridocyclitis should cause delay in the decision for surgery. Many corneas with chronic ulcers and necrosis inevitably have inflammation within the eye, and surgery often must be pursued in spite of its existence.

Those eyes already showing spread of an infection (bacterial or fungus) to the anterior chamber should be treated medically in an effort to control the inflammation prior to a consideration of surgery.

Selection of surgical procedure

The surgical procedure often can be selected on the basis of the corneal lesion itself, provided all other factors are favorable. Those patients with superficial chronic epithelial lesions often benefit by simple debridement, followed by patching of both eyes for several days. Patients with recurrent superficial epithelial and stromal herpes, if sufficiently persistent, are ideally treated by lamellar keratoplasty, as are those with superficial scars.

Those with disciform lesions in the stroma which have resulted in scarring usually have involvement of the deeper lamellae, and penetrating keratoplasty most often is indicated. The prognosis in such cases is excellent.

Those with chronic ulcers following typical herpetic infections which persist for a number of months most often benefit by lamellar keratoplasty, confined to the region

of the ulcerated area. Such keratoplasties may be superficial or deep, depending upon the lesion, and may be partial or total, depending upon its geographic extent. They have the advantage that they are easy to perform, result in healing of torpid lesions, and if unsuccessful from a visual standpoint, may be followed by further lamellar keratoplasty or a penetrating keratoplasty.

Severe scarring and vascularization of the cornea should not deter the surgeon in those patients with poor vision in both eyes, or even in one eye. Small repeated applications of X rays with the 50 kv. machine, affording tissue doses of 5 to 700 r, at times lead to decrease in vascularity and edema. Following this, total lamellar keratoplasty, replacing two thirds the thickness of the cornea, may lead to a more healthy cornea into which to place a penetrating graft at a later date.

Case reports

It is difficult to present results in tabular form. Therefore it is considered desirable in this small group to present my experiences in case report form. The following review presents the results of 22 keratoplasties in 18 patients suffering with various types of corneal herpetic affections.

Group I. Keratoplasty for disciform keratitis and other types of corneal leukomas

Case 1

Mr. R. C., aged 21 years, was with the American Armed Forces in France in December, 1945. He developed a dendritic keratitis of the right eye, which caused considerable scarring. He was invalided home and at the Army Hospital in San Francisco was found to have 20/50 vision in the right eye and 20/200 in the left. The left had always been partially amblyopic because of a high hyperopia and astigmatism.

Examination showed a diffuse scar in the stroma of the right cornea with vascularization of the superficial one third. The vision could not be improved with glasses. An optical iridectomy was done on May 5, 1947, at the U. S. Veterans Hospital, San Francisco. Very little visual benefit resulted. On January 11, 1949, there commenced what eventually proved to be a series of recurrent dendritic ulcers, extending to the summer of 1952.

Following the subsidence of an active lesion, a 7.0-mm. penetrating keratoplasty was done on August 21, 1952. A number of minor complications resulted in postoperative edema of the graft, persisting to December, 1952. In January, 1953, the corrected vision with a -8.75D. sph. $\bigcirc$ $+5.0$D. cyl. ax. 115° was 20/25. This refractive error and the visual acuity have persisted to the present date (August, 1956).

Case 2

Mr. J. B., aged 60 years, had bilateral dendritic ulcers of the corneas for a period of 10 years. The ulcers recurred each spring, presumably from exposure to sun and dust during the planting and irrigation of melons. Eventual visual disability resulted from scarring.

The vision in the right eye was 20/200 and in the left 15/200. There was bilateral superficial and deep corneal scarring and vascularization, posterior synechias, and secondary cataracts.

On February 8, 1950, a 6.0-mm. penetrating keratoplasty was done on the left eye, and the surgical result was excellent. Vision was 20/50 with a -9.0D. sph. on April 7, 1950. By April, 1951, vision had dropped to 20/200 in this eye as the result of increasing lens opacities.

During July, August, and September, 1951, three recurrences of dendritic keratitis occurred in the right eye, with severe iritis and hypopyon. By November the cornea was uninflamed. A penetrating keratoplasty of 6.0 mm. was done on the right eye December 4, 1951. Lens opacities were marked in this eye prior to corneal surgery. On January 15, 1952, vision in the right eye

with a −5.5D. sph was 20/200, and in the left with a −10.5D. sph. ⊃ +1.5D. cyl. ax. 125° was 20/200.

On February 19, 1952, an intracapsular lens extraction was done on the right eye. The graft became moderately edematous following surgery, but gradually cleared so that in early 1954 the right vision was 20/70 with correction. In July, 1954, a right retinal detachment occurred, but fundus details were hazy and a hole could not be located. Retinopexy was unsuccessful after two operations and vision was lost.

The left vision continued at 20/200 to July, 1956, when a recurrence of a dendritic ulcer occurred in the graft (fig. 1). Isolations were not attempted immediately, but the clinical findings were typical, and the graft became anesthetic.

In August, while the patient was under treatment with 1:10,000 quaternary ammonium chloride solution (Zephiran) during the day and merthiolate ointment at night, a pneumococcic ulcer of sluggish character appeared in the lower cornea outside the graft (fig. 2). At the present time the patient is still hospitalized and the ulcer in the graft is slowly clearing without severe scarring and the pneumococcic ulcer is slowly subsiding.

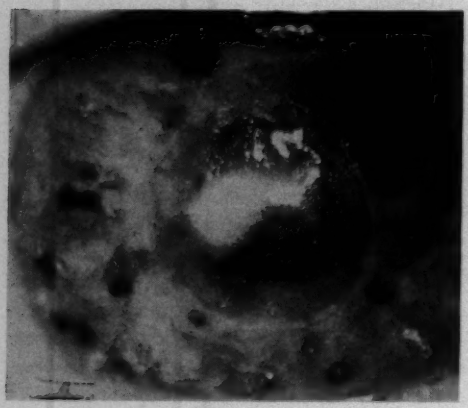

Fig. 1 (Hogan). Group I. Case 2. Recurrent herpes in corneal graft six and one-half years after operation.

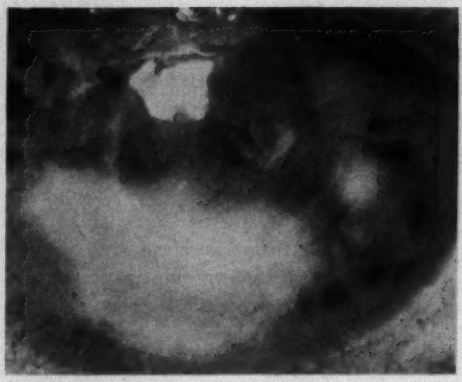

Fig. 2 (Hogan). Group I. Case 2. Probable recurrence of dendritic keratitis in a graft four years after operation. The lesion in the cornea below the graft is a pneumococcic ulcer which developed later.

CASE 3

Mr. W. L., aged 63 years, injured the left eye at the age of 37 years with a wire, leaving corneal scars. An optical iridectomy failed to improve vision. The right eye was amblyopic due to a refractive error.

Nine months after the injury he commenced having recurrent dendritic ulcers of the left cornea, eventually resulting in severe scarring and visual loss. The left vision in February, 1955, was hand movements and the right vision was 2/200. The left cornea was diffusely leukomatous and there was no active keratitis.

A 7.0-mm. penetrating keratoplasty was done on the left eye on February 24, 1955. On discharge, March 7, 1955, the graft was clear. On June 25, 1955, the graft remained clear, there were incipient lens changes, and the vision could not be improved to better than 20/200 with lenses. It was not determined by his physician why the acuity was so reduced, except that a macular lesion was suspected. On August 20, 1955, the graft was reported to have developed superficial clouding in spite of cortisone therapy.

CASE 4

Mr. W. H., aged 61 years, had normal vi-

sion in the left eye up to June, 1954. The left eye had poor vision since childhood, due to a refractive error. In June there developed a recurrent dendritic keratitis which was treated with cortisone, and healing occurred. It recurred in August, 1954, and caused stromal scarring with visual reduction to 10/200 (fig. 3).

On July 20, 1955, a 6.5-mm. penetrating keratoplasty was done on the left eye. The postoperative course was uneventful, and the graft remained clear. Vision was correctible to 20/60 with a +1.0D. sph. ⊂ +0.75D. cyl. ax. 82° through November, 1955. In December lens opacities developed and increased in severity so that in February, 1956, vision was 20/200. At the present time vision is 10/200 and a cataract extraction is contemplated.

Case 5

Mr. J. C., aged 40 years (University of California Clinics U219714), had recurrent herpetic ulcers of the right cornea for 10 years, leading to severe disciform keratitis and vascularization. Vision was reduced to counting fingers. The cornea was anesthetic.

On July 16, 1954, an 8.0-mm. penetrating keratoplasty was done on this eye. The graft remained edematous for three months. It gradually cleared, so that only a slight epithelial haze remained. Vision, however, could not be improved better than 20/200 with glasses. On January 2, 1955, an infiltrate appeared in the graft, with rapid ulceration. Cultures were positive for pathogenic Staphylococci, and by January 7th a large ulcer was present (fig. 4). Intensive therapy with antibiotic agents to which the organism was sensitive led to healing by March 1, 1955. The graft was severely scarred. A further keratoplasty is contemplated.

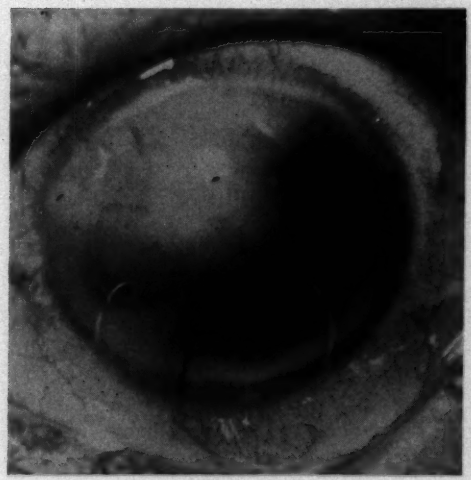

Fig. 3 (Hogan). Group I. Case 4. Preoperative. Herpes scar of cornea.

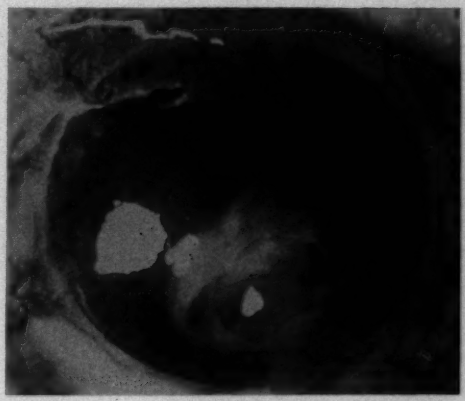

Fig. 4 (Hogan). Group I. Case 5. Late infection in corneal graft.

Case 6

Mr. T. H., aged 79 years, had failing left vision for 10 years as the result of recurrent herpetic ulcers with band keratopathy, and incipient cataracts. His keratopathy was treated with hydrochloric acid to remove the calcium. It led to a recurrence of the herpes keratitis and to further visual reduction.

After the inflammation had subsided a penetrating keratoplasty of 6.0 mm. was done on the left eye on November 19, 1954. The postoperative course was uneventful and the graft remained clear. On February 21, 1955, vision was 20/200 and could not be improved beyond this because of lens changes (fig. 5). Up to the present time

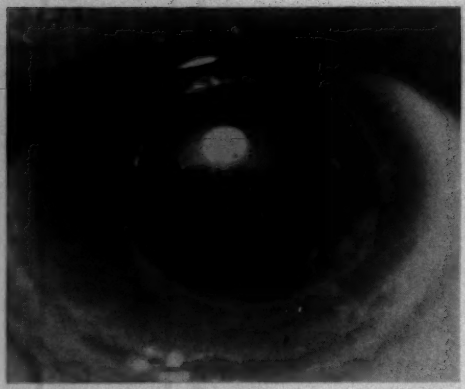

Fig. 5 (Hogan). Group I. Case 6. Postoperative transplant.

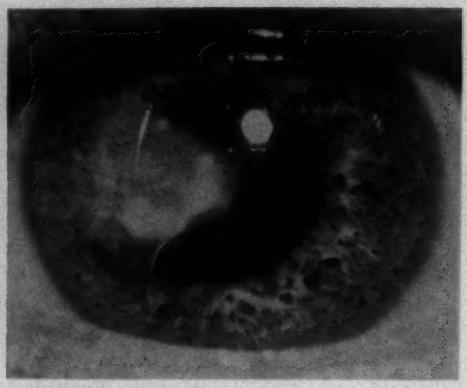

Fig. 6 (Hogan). Group I. Case 7. Preoperative herpes scar.

(1956) there has been no recurrence of the herpes.

CASE 7

Mr. C. B., aged 32 years, had an attack of typical dendritic keratitis in March, 1954, characterized by redness and pain in the left eye with marked visual reduction. Treatment was carried out for three months, and the lesion eventually healed, leaving a corneal scar, and vision of 20/400. After a period of two years the scar had cleared somewhat (fig. 6), with a manifest vision of 20/200. It could be improved to 20/70 with correction but this acuity was unstable and, in ordinary light, the average acuity was 20/200. The patient's occupation required a vision of 20/20 with correction, and he wished surgery with the chance of improvement.

A 6.0-mm. penetrating keratoplasty was done on May 14, 1956, and the patient had an uneventful postoperative course. On August 23, 1956, the manifest vision was 20/40, and improved to 20/30 with a moderate astigmatic correction.

Group II. Keratoplasty for recurrent herpetic ulcerative keratitis

CASE 1

Mr. P. C., aged 70 years (University of California Clinics U233072), was admitted on January 18, 1955, with a chief complaint of congestion, tearing, and visual loss for 18 days. A similar attack occurred in 1953, resulting in some visual loss.

Examination showed the vision to be counting fingers in the right eye and 20/50 in the left. There was an ulcer in the peripheral one third of the lower nasal right cornea, with vascularization from the adjacent limbus (fig. 7). The entire cornea was anesthetic. Herpes virus was not isolated from

Fig. 7 (Hogan). Group II. Case 1. Chronic herpetic corneal ulcer with vascularization.

the ulcer, and cultures otherwise were negative.

In view of the anesthesia, previous attack, and the course of the disease, a diagnosis of chronic herpes ulcer was made. By March, 1955, the lesion was almost well, and within another month complete healing occurred.

A recurrence of the ulcer developed in October, 1955, with iridocyclitis and hypopyon. All types of therapy failed.

A 7.0-mm. penetrating keratoplasty was done on April 23, 1956, without complication. The graft was clear at the time of discharge on April 29, 1956. Two days later the patient returned with a hazy graft but without other symptoms. On May 3rd there was a necrotic ulcer in the graft with a hypopyon (fig. 8). A staphylococcic infection of the anterior segment ensued, necessitating enucleation on May 20, 1956.

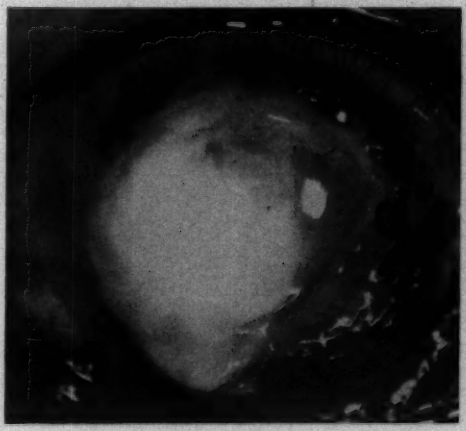

Fig. 8 (Hogan). Group II. Case 1. Ten days, postoperative infection.

Case 2

Miss M. G., aged 37 years, had almost continuous recurrences of dendritic keratitis since the age of five years. During the period from 1952 to 1954 the recurrences were so frequent in the right eye that the patient was prevented from carrying out her work efficiently. She was first seen on July 8, 1954,

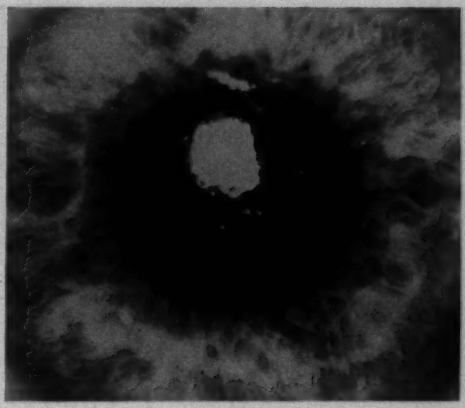

Fig. 9 (Hogan). Group II. Case 2. Preoperative recurrent herpes.

with an active ulcer in the right eye (fig. 9). Therapy was not successful so that on August 27, 1954, a lamellar keratoplasty was done (8.0-mm. diameter, fig. 10). The postoperative course was uneventful and the graft remained clear. Vision was 20/200 with the best possible correction (the patient stated she had always been partially amblyopic in this eye). She was well until October, 1955, when a small round nonherpetic infiltrate (deep and yellow; approximately 1.0-mm. diameter) appeared in the cornea below the graft. It healed quickly, and there

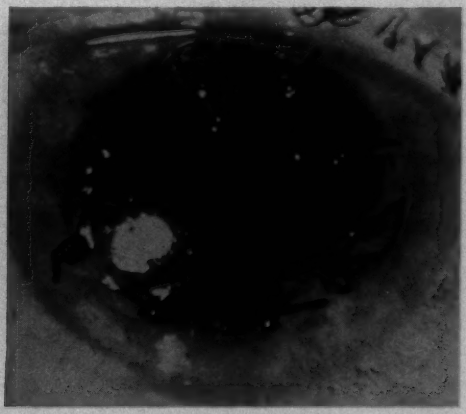

Fig. 10 (Hogan). Group II. Case 2. Lamellar keratoplasty for recurrent and chronic herpetic ulcers.

have been no recurrences to date (August, 1956).

CASE 3

Mr. F. H., aged 63 years, had recurrent left corneal ulcers for 10 years, resulting in markedly blurred vision. In November, 1954, a severe recurrence developed, increased in size and depth on treatment with cortisone, and resulted in a deep ulcer (fig. 11). Vision was reduced to light perception. All types of treatment, including debridement, failed to result in healing.

On April 6, 1955, a 6.5-mm. penetrating keratoplasty was done. Postoperative synechias of the iris developed at the 6-o'clock position but the graft was clear. On April 27th a synechiotomy was done. In July the vision was 20/100 and the lower graft remained moderately edematous. In February, 1956, the graft was clear, and vision continued at 20/100 (fig. 12). Diffuse lens changes were observed which caused the visual reduction.

CASE 4

Mr. P. J., aged 65 years, will be reported in brief because the surgical prognosis was poor from the outset. He developed a dendritic ulcer, which was treated with cortisone, resulting in extension into the stroma, chronic ulceration, and eventual perforation (fig. 13). It was hoped that Diamox, a keratoplasty, and corticosteroids might offer a slight chance of saving the eye. Glaucoma followed the surgery, and was not controlled with any type of treatment. Enucleation became necessary.

Fig. 12 (Hogan). Group II. Case 3. Postoperative transplant.

CASE 5

Miss P. D., aged 13 years, suffered severe recurrent herpetic infections of the right eye commencing at the age of seven years and continuing to 1954. During one attack in

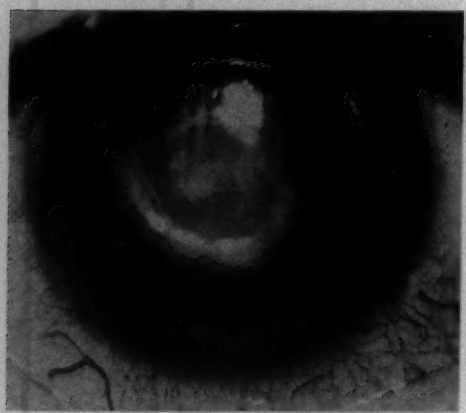

Fig. 11 (Hogan). Group II. Case 3. Chronic herpetic ulcer.

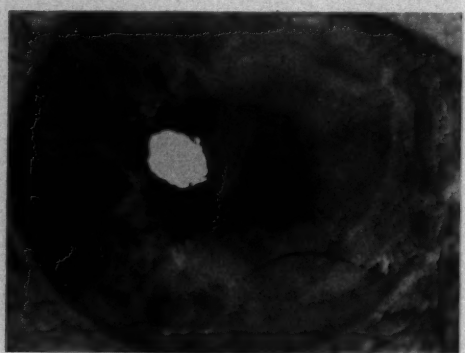

Fig. 13 (Hogan). Group II. Case 4. Descemetocele preoperatively.

1954 which lasted many months, there developed a chronic ulcer and severe iritis, posterior synechias, and lens changes (fig. 14). On December 29, 1954, a 6.0-mm. penetrating keratoplasty was done on this eye. Three days later an iris prolapse followed a severe sneezing spell. It was excised but the anterior chamber remained shallow on the nasal side, resulting in formation of synechias. The graft was fairly clear, however, so nothing was done. Vision was 20/200 in July, 1955. In November, 1955, the nasal side of the graft was somewhat edematous, but the patient had no symptoms. Vision was 20/100 and could not be improved with a lens (fig. 15). For a number of reasons nothing further was done.

Fig. 15 (Hogan). Group II. Case 5. Postoperative transplant.

CASE 6

Mrs. R. S., aged 67 years, developed a herpes ulcer of the left cornea in 1953, requiring three months to heal. It recurred on May 1, 1956. Therapy with sulfonamides and cortisone failed to effect healing. A persistent ulcer developed, and all therapy designed to result in healing failed (fig. 16). Vision was reduced to hand movements. In view of the persistence of the ulcer it was decided to perform a lamellar keratoplasty, which was done on June 26, 1956. The postoperative course was uneventful, the cornea remaining entirely clear to the present time (August, 1956), and visual acuity is 20/200, correctible to 20/50 with a lens.

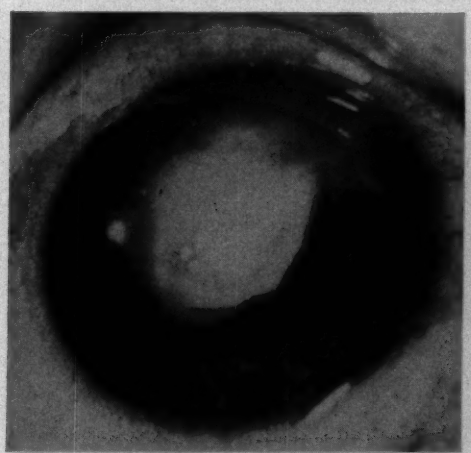

Fig. 14 (Hogan). Group II. Case 5. Preoperative transplant.

CASE 7

Mr. J. S., aged 65 years, developed a herpetic infection of the left cornea in September, 1952, resulting in scarring but not seriously affecting the vision. The ulceration recurred several times up to January, 1954, at which time vision was reduced to 20/200 and the pupillary area was involved by an ulcer. Because of the persistence of ulceration, a 7.0-mm. lamellar keratoplasty was

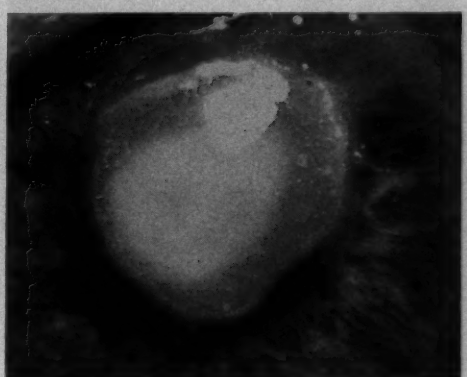

Fig. 16 (Hogan). Group II. Case 6. Preoperative.

done on February 17, 1954. The postoperative course was uneventful. Vision corrected to 20/70 (August, 1956), and there has been no recurrence of the herpetic infection.

Group III. Keratoplasty for disciform ulcers

CASE 1

Mrs. J. S., aged 37 years, was a housewife. At the age of nine months, this patient developed ulcers on both corneas which resulted in scarring and visual reduction. It was considered she might have had an old interstitial keratitis, although the possibility of an early herpes infection could not be ruled out.

In early July, 1953, the patient developed a keratoconjunctivitis of the left eye, with associated iritis and secondary glaucoma. Therapy with cortisone, atropine, and paracentesis did not result in subsidence of symptoms. After a month gradual clearing occurred. In September, 1953, the patient developed a typical dendritic ulcer of the left cornea, with secondary glaucoma. The inflammation and elevated tension were controlled by October, 1953, but a disciform lesion had developed. Recurrent ulcers occurred around the disciform lesion in the central cornea (fig. 17). In January, 1954, a small perforation occurred at the nasal edge of the disciform lesion (fig. 18). A 7.5-mm. penetrating keratoplasty was done on February 23, 1954. The postoperative course was uneventful for four days when an iris prolapse occurred on the nasal side, requiring repair. Following this the postoperative course was normal. The graft remained hazy and thickened through August, 1954, but there were no symptoms, and the epithelium was not edematous (fig. 19). There has not been a recurrence of the herpes in the graft up to the present time (August, 1956), but vision is only hand movements.

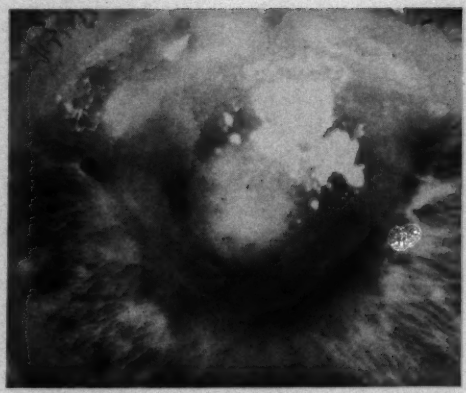

Fig. 18 (Hogan). Group III. Case 1. Preoperative. Chronic herpes ulcer.

CASE 2

Mr. R. P., aged 43 years, developed a herpetic infection of the right cornea in

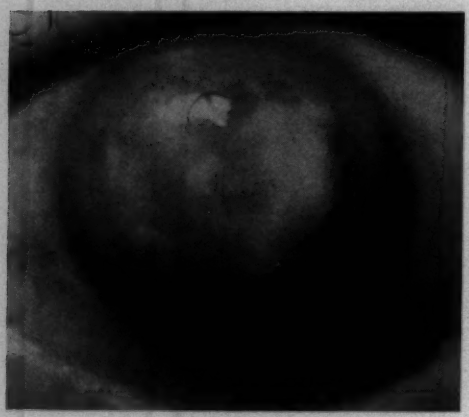

Fig. 17 (Hogan). Group III. Case 1. Preoperative. Chronic herpes ulcer.

Fig. 19 (Hogan). Group III. Case 1. Four months after operation.

1929, when he was aged 18 years. In September, 1953, it recurred, and had persisted up to the time he was referred in May, 1954. At that time he had a leaking necrotic ulcer in the central cornea with considerable surrounding edema. A history of treatment with hydrocortisone for many months was given. Cultures were negative, and a virus was not isolated following the scraping of the crater of the ulcer.

On May 20, 1954, a 6.0-mm. penetrating keratoplasty was done. The temporal chamber became shallow five days later, and synechias developed. It finally became necessary to repeat the grafting in October, 1954, and again in November, 1955 (fig. 20). At the present time (August, 1956) vision is 20/200 and the graft is edematous (fig. 21).

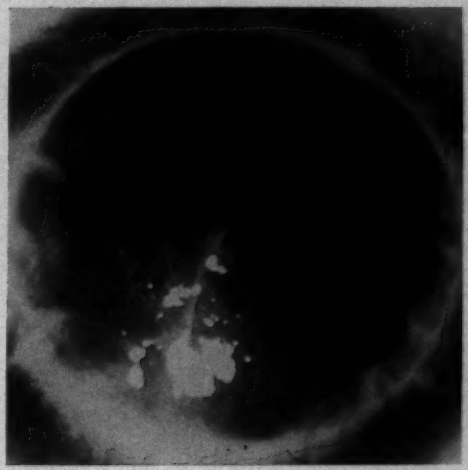

Fig. 21 (Hogan). Group III. Case 2. Third postoperative transplant.

CASE 3

Mrs. S. H., aged 65 years. This unfortunate lady developed bilateral dendritic ulcers of the corneas in March, 1953. She was treated with cortisone topically and with antibiotics by mouth. A monilia ulceration developed in the right cornea and it perforated. The ulcer eventually healed but by this time the cornea was thickened and scarred and the tension was elevated. Vision was reduced to poor light perception and projection. The left cornea showed a dense leukoma with marked vascularization. The tension seemed to be normal and vision was good light perception and projection. A keratectomy was done on the left eye on April 6, 1953, with a good result (fig. 22). On June 26, 1954, a 7.0-mm. penetrating keratoplasty was done, without complication. On July 20th, a staphylococcic conjunctivitis developed, with secondary involvement of the lower graft, causing it to become completely cloudy. On September 9, 1954, a 7.5-mm. penetrating keratoplasty was repeated on this left eye without success.

CASE 4

Mr. D. Y., aged 83 years, had bilateral

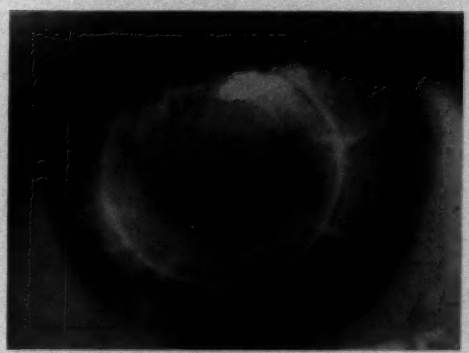

Fig. 20 (Hogan). Group III. Case 2. Postoperative transplant.

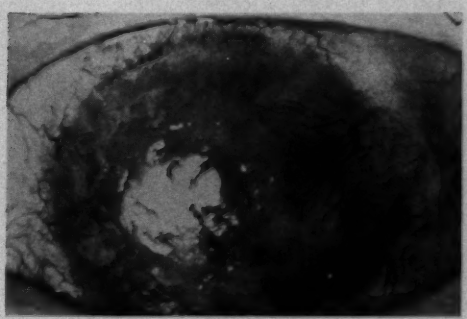

Fig. 22 (Hogan). Group III. Case 3. Postkeratectomy.

immature cataracts causing visual reduction to 20/80 in both eyes. In June, 1955, a herpes infection developed in the right cornea and resisted all forms of treatment (fig. 23). Treatment with cortisone had been carried out.

Chronic ulceration, necrosis, and diffuse keratitis eventually led to a decision to perform a keratoplasty. This was done on May 27, 1956, a 6.0-mm. penetrating keratoplasty being done. The postoperative course has been uneventful, the graft is somewhat edematous, and the patient comfortable. Vision is 10/200 (August, 1956).

Comment on surgical cases

In retrospect it must be concluded that the utilization of penetrating keratoplasty on extensive deep herpetic keratitis with ulceration leaves much to be desired. It is now our opinion that a preliminary lamellar keratoplasty is the procedure of choice.

Lamellar keratoplasty should be utilized for superficial to moderately deep herpetic scarring of the corneal stroma. Penetrating keratoplasty is eminently successful in patients with disciform and deep scars.

Pathologic considerations

The pathology of the excised and diseased tissue has been of great interest to our group, because we have been surprised at the degree of inflammation exhibited by apparently quiet corneas. Several corneas removed at least six months after subsidence of active inflammation exhibited considerable lamellar necrosis, edema, and interlamellar round cell infiltration, even though the inflammation appeared to be entirely quiet clinically (fig. 24). Those excised corneas which had exhibited active ulceration of a chronic and recalcitrant type showed considerable necrosis of the lamellae, with vascularization (fig. 25). The corneas removed from those cases having diffuse disease, with necrosis and vascularization, invariably showed widespread lamellar destruction, intense round cell infiltration and vascularization. Up to the present writing fungi were not found in any of this group, either culturally or by special stains.

Fig. 23 (Hogan). Group III. Case 4. Preoperative chronic herpes.

Fig. 24 (Hogan). Keratitis in an apparently quiet cornea.

Bacteriologic studies

Excised, inflamed, and ulcerated corneal tissue from this group of herpes cases was investigated for the presence of virus in two cases. The excised tissue was ground and inoculated on the chorioallantoic membrane,

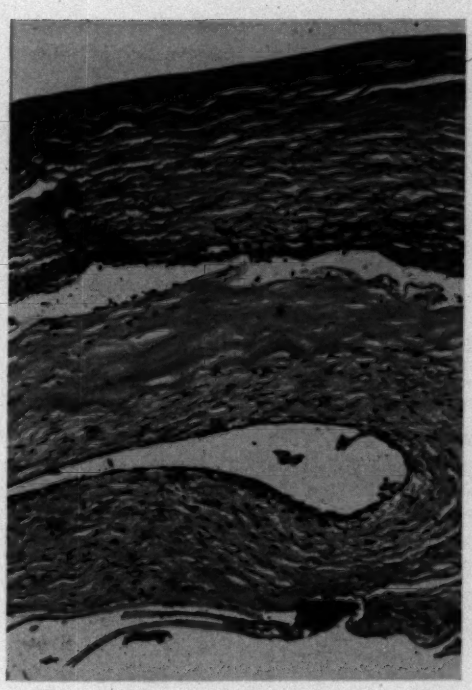

Fig. 25 (Hogan). Keratitis with necrosis.

on fresh tissue cultures of HeLa cells, and on ordinary media. There have been no isolations of herpes virus, or other organisms. It is interesting to speculate as to the lack of isolation of a virus from this group of postherpetic cases. The following possibilities exist:

1. The virus is present in the corneal stroma, but cannot be isolated because of the concomitant masking effect of antibody.

2. The virus is not present, and the corneal lesions are due to trophic changes as a result of nerve damage.

3. The virus is present in such low concentration that it cannot be isolated.

Discussion

A. Technical surgical considerations

This group of herpes infections of the cornea has been the most interesting of all our cases which have been subjected to corneal transplantation. Those with healed disciform scars which had not cleared within a reasonable time underwent penetrating keratoplasties, and ordinarily had few sequelae or complications. The same may be said for those with less localized scars due to recurrent dendritic keratitis. I have generally preferred penetrating keratoplasties for these two groups unless the scars were superficial in position, although at the present time we prefer lamellar keratoplasty for those scars affecting the superficial half to two thirds of the stroma.

The cases with chronic or recurrent superficial ulceration associated with a surrounding keratitis have also done well. Penetrating keratoplasties have been done for the most part, although lamellar keratoplasty may have given similar results. Those corneas showing deeper ulceration with surrounding necrosis were considered to have a better prognosis following penetrating keratoplasty, although I now feel it is better to do a preliminary lamellar keratoplasty.

The patients with diffuse keratitis, vascularization, and scattered areas of stromal necrosis did poorly with penetrating keratoplasties, and it is most likely that lamellar keratoplasty should have been attempted as a primary procedure, followed if necessary by penetrating keratoplasty at a later date.

B. Recurrence of herpes in grafted corneas

It was our hope that lamellar or pentrating keratoplasty might prevent the recurrence of ulceration, or so alter the local conditions that the disease would be eradicated. For the most part, the results have been gratifying, for only one patient (six and one-half years after surgery) has had a recurrence of dendritic ulceration in a grafted cornea (Group I, Case 2). A number of other cases had almost constant recurrences and have been free of attacks since (Group II, Cases 2 and 5).

Summary and Conclusions

1. Keratoplasty is a satisfactory procedure

for the treatment of diffuse and disciform scars of the cornea due to herpes infections, chronic postherpetic ulcers, and persistent postherpetic keratitis.

2. Penetrating keratoplasty is more desirable in patients with disciform keratitis and deep chronic postherpetic ulceration. Lamellar keratoplasty seems to give better results in those eyes having diffuse superficial scars, diffuse keratitis with vascularization, and in the more shallow types of ulceration. It has the advantage that it can be repeated, or be followed by penetrating keratoplasty at a later date.

3. Pathologic investigations of corneas excised for apparently inactive herpes showed considerable persistent keratitis.

4. Corneal transplantation offers hope to patients with recurrent herpetic infections that their disease will be completely eradicated.

5. A virus was not isolated from the tissues of two corneal grafts removed at keratoplasty.

University of California
Medical Center (22).

References

1. Franceschetti, A., and Maeder, G.: Indications for corneal transplants and selection of cases. Textbook on Corneal Grafts (B. W. Rycroft, ed.). St Louis, Mosby, 1955, pp. 76-85.
2. Sanders, T. E., and Ley, A. B.: Communication to A.M.A. Sec. Ophth., June, 1956.

Summary of the Symposium

It is hoped that this first joint meeting of ophthalmologists, virologists, and epidemiologists, called by two Study Sections of the National Institutes of Health to consider the problem of viral keratoconjunctivitis, may serve to stimulate the calling of other joint meetings at which workers in several disciplines may discuss problems of common interest and plan further investigative effort.

The following is an attempt to summarize briefly the principal areas of agreement and of difference among the workers in this field as brought to light in the course of the symposium, and to pinpoint the unsolved problems upon which further work must be done.

Pharyngoconjunctival fever

Clinical picture

The symposium focused attention on the clinical features of pharyngoconjunctival fever (PCF), particularly with respect to corneal changes. There was general agreement among the ophthalmologists that the conjunctival manifestation is an acute follicular conjunctivitis without pseudomembranes, characterized by a scanty, predominantly mononuclear cell exudate, an associated mild preauricular adenopathy, and a short course. It was agreed that the disease is clinically indistinguishable from the conjunctivitis of Newcastle disease or the acute follicular conjunctivitis known as "acute follicular conjunctivitis, Béal."

There was some difference of opinion on the frequency and nature of the corneal changes of pharyngoconjunctival fever but agreement that the original descriptions of the disease by Bell, Rowe, Engler, Parrot, and Huebner, and by Ryan, O'Rourke, and Iser, which stated that no corneal changes occurred, must now be modified. The University of California group maintained that the corneal changes were primarily epithelial, with only a very occasional transient subepithelial infiltrate, and that they bore little resemblance to the prominent, often grossly visible subepithelial infiltrates of epidemic keratoconjunctivitis. The University of Toronto group, on the other hand, reported that some of their cases of pharyngoconjunctival fever had lesions indistinguishable from those of epidemic keratoconjunctivitis.

There was general agreement among both ophthalmologists and virologists that, although sporadic cases might offer a problem in differential diagnosis, under epidemic conditions pharyngoconjunctival fever could be readily distinguished from epidemic keratoconjunctivitis. Huebner, for example, on the basis of extensive experience with pharyngoconjunctival fever, said that he had never seen a case of typical epidemic keratoconjunctivitis and that none of the inoculations made by the National Institutes of Health group with adenovirus 3 had resulted in a condition resembling epidemic keratoconjunctivitis. Ryan stated that he had only once seen a case of pharyngoconjunctival fever with keratitis and that it was a transient manifestation. It was recalled that Cockburn, who is not an ophthalmologist, had no difficulty distinguishing cases now known to have been pharyngoconjunctival fever from the cases of epidemic keratoconjunctivitis he saw in the Kansas City epidemic; and that during the 1950 and 1951 epidemics of pharyngoconjunctival fever in children that occurred in the San Francisco Bay region, epidemic keratoconjunctivitis was not considered in the differential diagnosis because of the absence of typical corneal lesions.

There seemed to be agreement that pharyngoconjunctival fever had existed in the United States prior to World War II but that epidemic keratoconjunctivitis was probably introduced in 1941 in connection with the war effort.

There was general agreement as to the identity, both clinical and serologic, of pharyngoconjunctival fever and the conjunctivitis described by Cockburn under the name "Greeley conjunctivitis." In connection with the suggestion that the so-called "acute follicular conjunctivitis, Béal" might also be identical with pharyngoconjunctival fever, it was recalled that in about one-third of Béal's original cases there was associated upper respiratory disease. Béal made no reference to swimming-pool transmission but later observers have done so. The finding of corneal changes in Béal's conjunctivitis has not been reported, however, and further work must be done before certain identification of the two diseases can be made. It may be that corneal changes have not been reported because of incomplete slitlamp studies.

Epidemiology

All observers have noted apparent transmission of pharyngoconjunctival fever through swimming pools and the highest incidence of the disease in the summer months. In discussion, however, Huebner questioned the role of the swimming pool as a transmitter of the virus, suggesting rather that swimming tended to traumatize the conjunctiva and thus to favor infection which might otherwise be resisted. This factor, of importance in epidemiology, certainly needs further study, particularly as to the survival of adenovirus in chlorinated and unchlorinated water. It is noteworthy that no one has reported the occurrence of office epidemics of pharyngoconjunctival fever. This is in marked contrast to the high incidence of epidemics of epidemic keratoconjunctivitis in offices, hospitals, and dispensaries. This difference and the apparent predilection of pharyngoconjunctival fever for children could be explained by the high incidence of antibody to type 3 adenovirus in the general population and the low incidence of antibody to type 8.

Etiology

While it is clearly established that adenovirus type 3 is the usual cause of epidemic pharyngoconjunctival fever in children, other types have been encountered in sporadic cases and family outbreaks. These include types 2, 3, 6, and 7. There was general agreement on the frequent association of certain types of adenovirus with certain disease pictures, as well as on the ability of many types to produce similar pictures.

Epidemic keratoconjunctivitis

Clinical picture

There was general agreement among the symposium participants that the main features of epidemic keratoconjunctivitis consisted of (1) an acute follicular or pseudomembranous conjunctivitis, depending on the severity of the disease, with adenopathy; and (2) a keratitis in the form of typical round subepithelial infiltrates, with onset a week or 10 days after the onset of the conjunctivitis, and of long duration. It was agreed that the conjunctival changes are usually much more severe and long lasting than those of pharyngoconjunctival fever; in the latter, for example, pseudomembranes never have been observed. In a paper contributed to the symposium by Pillat, epidemic keratoconjunctivitis is clearly differentiated from nummular keratitis, a disease common in Europe but seen rarely, if ever, in the United States.

It was maintained by the University of California group that the corneal changes of epidemic keratoconjunctivitis are readily distinguishable from those of pharyngoconjunctival fever on the following grounds: (1) That the infiltrates of epidemic keratoconjunctivitis are usually grossly visible while those of pharyngoconjunctival fever are usually visible only with the slitlamp; (2) that the lesions of epidemic keratoconjunctivitis are primarily subepithelial while

those of pharyngoconjunctival fever are primarily epithelial; and (3) that when corneal changes occur in pharyngoconjunctival fever they appear at the same time as the conjunctival changes, while in epidemic keratoconjunctivitis they are delayed from seven to 10 days. Another difference mentioned was that permanent visual damage is known to have been caused occasionally by epidemic keratoconjunctivitis but never by pharyngoconjunctival fever.

It was agreed that an apparent predilection for children has been shown by pharyngoconjunctival fever but not by epidemic keratoconjunctivitis. It was further agreed that except in children epidemic keratoconjunctivitis is usually not associated with systemic signs of any kind. This is in marked contrast to pharyngoconjunctival fever which is regularly associated with systemic signs. According to Dr. Tanaka's report, however, epidemic keratoconjunctivitis in children has displayed severe systemic signs consisting of high fever, pharyngitis, otitis media, diarrhea, and vomiting. She refers to Mitsui's experiments in which transmission of material from this infantile form of the disease produced typical epidemic keratoconjunctivitis without such systemic signs in adults. If this transmission experiment had not been made, one would be tempted to believe that the children had had primary herpetic keratoconjunctivitis which is known to be accompanied by severe systemic signs and in which the conjunctival signs are similar to those of epidemic keratoconjunctivitis. Some of these children have been shown to have rises in adenovirus type 8 antibody.

Noteworthy in Dr. Tanaka's report is her statement that, until recently, epidemic keratoconjunctivitis and pharyngoconjunctival fever have been confused in Japan. This is reflected particularly in the reports of swimming-pool transmission. Further study is definitely indicated to determine how frequently epidemic keratoconjunctivitis spreads through swimming pools.

Epidemiology

All participants in the symposium agreed that epidemic keratoconjunctivitis is highly communicable and pharyngoconjunctival fever somewhat less so. It was brought out that in the United States, swimming-pool transmission has been typical of pharyngoconjunctival fever but not of epidemic keratoconjunctivitis, that transmission in hospitals, dispensaries, and private offices has been characteristic of epidemic keratoconjunctivitis but unknown with pharyngoconjunctival fever, and that epidemic keratoconjunctivitis has had a marked tendency to spread among professional personnel and pharyngoconjunctival fever has not. In the San Francisco Bay area, for example, two sporadic cases of pharyngoconjunctival fever in general physicians were studied, but in the course of two major epidemics of the disease, primarily among children, in this same area, no instance of its transmission to ophthalmologists or pediatricians was noted in spite of the many hundreds of cases that developed. Epidemic keratoconjunctivitis, on the other hand, spreads with ease through hospitals, and so forth, and has affected numerous ophthalmologists. That it can be a serious public health problem was re-emphasized by Dr. Leopold's report of a recent hospital epidemic among postoperative patients.

The role of trauma, particularly industrial trauma, and the connection between foreign-body removal and epidemic keratoconjunctivitis, were discussed at length. It was interesting that in the Schenectady epidemic Dr. Korns found no significant difference in the opacities or clinical severity of cases apparently contracted in the eye clinic after foreign-body removal, and of cases infected subsequently as a result of household contacts. In an unpublished study of 32 ophthalmologists who contracted the disease while treating patients, it was found that all had corneal opacities and that the only conceivable trauma had been the simple rubbing of the eyes with the fingers. In a number of

these cases the disease was very severe. The role of trauma in transmission and its effect on the severity of the disease and the incidence of opacities are certainly still in doubt.

In none of the studies of epidemic keratoconjunctivitis and pharyngoconjunctival fever have any instances of a second infection with either disease in the same individual been noted. Apparently both diseases confer a definite and lasting immunity.

Etiology

Whether any type of adenovirus except type 8 has been found associated with typical epidemic keratoconjunctivitis is open to some doubt. The evidence listed below supports the contention that adenovirus type 8 is at least one agent capable of causing the picture typical of epidemic keratoconjunctivitis. Some confusion has arisen because certain observers have recorded keratitis of minor degree and short duration in the course of adenovirus type 3 and type 7 infections. Considering the spectrum of any disease caused by a specific etiologic agent, it appears natural that in addition to typical full-blown cases there will be many marginal ones lacking certain diagnostic features. The typical cases are the only ones that can be diagnosed initially until the etiologic agent has been established. When this has been accomplished, atypical cases can be diagnosed by laboratory means. At the present time the evidence suggests that types of adenovirus other than type 8 have not given rise to the typical diagnostic subepithelial opacities, and that overlapping therefore exists mainly between atypical epidemic keratoconjunctivitis (associated with adenovirus type 8) and transient keratitis seen with infections due to other types.

The evidence pointing to adenovirus type 8 as at least one of the etiologic agents of typical epidemic keratoconjunctivitis can be summarized as follows:

1. Three strains of adenovirus type 8 have been isolated to-date, all from eye lesions. This type of adenovirus has not been recovered from other sources. Adenovirus type 8 differs biologically from other adenovirus types, particularly in its exceedingly low infective titer for tissue culture cells compared to the relatively large amount of noninfective virus (antigenic material) present.

2. Of 70 patients with the definite clinical diagnosis of epidemic keratoconjunctivitis in the United States, Canada, Switzerland, Italy, and Japan during 1951-55, 66 or 94.3 percent had neutralizing antibodies to adenovirus type 8 in a serum dilution of 1:10 or greater. Of 140 individuals from the same geographic areas and similar age groups who did not have clinical epidemic keratoconjunctivitis, only 10 or 7.1 percent had such antibodies. In an epidemic in Austria in 1952-53 from which sera were collected three years later, the incidence of 1:10 antibodies to adenovirus type 8 was 52.4 percent in epidemic keratoconjunctivitis and 13.3 percent in controls.

3. Of 17 adequate paired sera available from patients with typical epidemic keratoconjunctivitis in Chicago, California, and Japan, 15 developed a fourfold or greater rise in neutralizing antibodies to adenovirus type 8 during their illness. Unfortunately, there was much variation in the highest titers reached by these individuals and in the length of time such antibody titers persisted. In all individuals followed through the course of adenovirus type 8 infection of the eye, the antibody titer did not exceed 1:20 two years after onset of the infection. Thus retrospective serologic investigations are virtually impossible, unless sera from typical cases have been obtained within one year of onset and properly stored thereafter.

4. Patients with epidemic keratoconjunctivitis who exhibited a marked antibody titer rise against adenovirus type 8 did not have such titer rises for other types of adenovirus, for herpes simplex virus, or for the "E.K." strain of St. Louis encephalitis virus.

5. Adenovirus type 8 was inoculated into one eye of each of five volunteers. Four de-

veloped typical epidemic keratoconjunctivitis; the fifth had specific antibodies to adenovirus type 8 at the time of inoculation and remained well. The uninoculated eye became involved in three volunteers six to eight days after the inoculated eye. Adenovirus type 8 was re-isolated from the lesions and the volunteers developed significant antibody titer rises. Work is currently in progress to establish whether vaccination of volunteers with adenovirus type 8 will prevent the development of epidemic keratoconjunctivitis upon subsequent inoculation of the eye with the agent.

The position of the so-called "Sanders virus" of epidemic keratoconjunctivitis was discussed at length. This agent was originally implicated in the epidemic keratoconjunctivitis epidemics of 1940-42. At that time it was isolated repeatedly by Dr. Murray Sanders from typical cases and gave serologic reactions with these patients' sera. The subsequent fate of this virus is not known, but in 1947 the agent carrying the label of "EKC virus"—perhaps derived from the original isolation—was a variant of St. Louis encephalitis virus and had no serologic relationship to the epidemic keratoconjunctivitis cases which occurred between 1947 and 1955. While there was much speculation concerning this confusing situation, no final solution to the riddle was forthcoming. It was felt that an exact repetition of the efforts of Dr. Sanders in 1940 was not indicated because of the high probability that in repeated animal passage a latent virus would be "picked up" accidentally. This has already occurred in the hands of some workers. Thus it was concluded that (a) the nature and fate of the original "Sanders virus" remains uncertain, and (b) the strains of that virus currently available bear no evident relationship to the disease epidemic keratoconjunctivitis as it exists at the present time.

The following points should be pursued in order to establish the etiology of epidemic keratoconjunctivitis on firm ground:

1. In epidemics occurring in various parts of the world, *entirely typical* cases should be studied by virus isolation and antibody titer determinations.

2. Additional volunteers should be inoculated with the recovered agents, their clinical and serologic responses should be noted, and the viral agent re-isolated.

3. In serologic surveys, the incidence of specific antibodies to isolated agents should be compared in *typical* and *atypical* cases diagnosed as epidemic keratoconjunctivitis, in other eye diseases, and in comparable control populations.

4. The agents isolated in various parts of the world should be exchanged by different laboratories in efforts to obtain confirmation of results.

Herpetic keratitis and keratoconjunctivitis

There was general agreement that the problems of herpetic keratitis and conjunctivitis have become more important since the war and since the introduction of cortisone and related steroids for topical use in ocular infections.

There seemed to be general agreement that primary herpetic infection of the eye may occur in children as a severe keratoconjunctivitis but that, in the secondary forms, the conjunctiva is usually spared and the cornea alone involved. It was also agreed that herpetic keratitis appears typically as a dendritic keratitis but that a variety of other superficial and deep forms can occur, and that of these the most important is the so-called "hypopyon keratitis" whose exact nature needs elucidation. The consensus was that the majority of cases of hypopyon develop as a result of secondary bacterial or mycotic infection, but the University of California group felt that, at least in certain cases, the action of herpes simplex virus alone is responsible. Further investigation of the non-dendritic forms of herpetic keratitis is much needed.

There was discussion of the possibility that some cases of dendritic keratitis in adults may represent primary infection. In view of

the paucity of available evidence, however, no conclusion could be reached. The problem needs further serologic work.

The role of steroid therapy in the development of chronicity and other complications in herpetic keratitis was then discussed. There seemed to be general agreement that the widespread topical use of the steroid hormones has contributed to the relatively high frequency of complications noted in the postwar years. Further experimental work is needed to determine whether this effect is due to increased proliferation of virus in the tissues or to an unfavorable effect on the tissues themselves.

Among the many questions that were raised and left unanswered concerning immunity in herpetic keratitis and conjunctivitis, two of the more important were:

1. Does the presence of antibodies protect the conjunctiva from involvement in corneal relapses?

2. When a conjunctivitis occurs in a relapse, as occasionally happens, does this always indicate the presence of secondary infection with bacteria or other agents?

It was agreed that herpes-simplex virus could be isolated with great ease from typical epithelial corneal lesions early in the disease, but only with great difficulty and infrequently from stromal lesions, and that it is isolated very infrequently in the absence of clinical herpetic disease. Its possible presence in the "latent stage" in the eye could thus lead to confusing laboratory results. The meaning of "latency" in herpes simplex and the problems concerned with the unmasking of latent viruses were discussed in detail.

Many questions dealing with primary infection versus recurrence were also examined. Does a recurrence always indicate that the same tissue area was involved in the primary infection? May a dendritic keratitis occurring for the (apparently) first time late in life be a primary infection, or is it always a recurrence of an infection 20 or 30 years before? In "latency" is the virus in an incomplete ("provirus") form requiring activation by the trigger mechanism to become infective, or is there infective virus present within certain cell groups which spreads when the close symbiotic relationship is disturbed by the trigger? Is the infection with herpes virus so strictly limited to the corneal epithelium that its total removal by curettage, or in the course of corneal grafting, may lead to permanent security from relapse?

The lack of unanimity of opinion on these questions suggested an urgent need for further investigative work.

Phillips Thygeson.
Ernest Jawetz.

AMERICAN JOURNAL OF OPHTHALMOLOGY

Published Monthly by the Ophthalmic Publishing Company

Address original papers, other scientific communications including correspondence, also books for review to *Dr. Derrick Vail, 700 North Michigan Avenue, Chicago 11, Illinois;* Society Proceedings to *Mrs. Katherine F. Chalkley, Lake Geneva, Wisconsin.* Manuscripts should be *original copies,* typed in *double space,* with wide margins.

Exchange copies of the medical journals should be sent to *Dr. F. Herbert Haessler, 561 North 15th Street, Milwaukee 3, Wisconsin.*

Subscriptions, application for single copies, notices of changes of address, and communications with reference to advertising should be addressed to the *Manager of Subscriptions and Advertising, 664 North Michigan Avenue, Chicago 11, Illinois.* Copy of advertisements must be sent to the manager by the 10th of the month preceding its appearance.

Change of address notice should be received not later than the 10th of the month prior to the issue for which the change is to go into effect. Both old and new addresses should be given.

Author's proofs should be corrected and returned within forty-eight hours to the *Manuscript Editor, Mrs. Katherine F. Chalkley, Lake Geneva, Wisconsin.* Fifty reprints of each article will be supplied to the author without charge. Additional reprints may be obtained from the printer, the George Banta Company, Inc., 450-458 Ahnaip Street, Menasha, Wisconsin, if ordered at the time proofs are returned. But reprints to contain colored plates must be ordered when the article is accepted.

OBSERVERS

Clark, W. H., *State of California Department of Health*
Crocker, T. T., *University of California*
Davenport, F. M., *University of Michigan*
Kempe, C. H., *University of California*
Langmuir, A. C., *Communicable Disease Center, U.S.P.H.S.*
Lowrey, Col. A., *Ocular Research Unit, Walter Reed Army Medical Center*
Marsh, L. J., *Division of Indian Health*
Minou, A. A., *World Health Organization fellow, Shiraz, Iran*
Reinhard, K., *Arctic Health Research Center*
Ryan, R. W., formerly *National Institutes of Health*
Members of the *Microbiology and Immunology Study Section:*
Chairman, Edwin H. Lennette
Executive secretary, Berwin A. Cole
Members of the *Sensory Diseases Study Section:*
Chairman, Phillips Thygeson
Executive secretary, Thomas E. O'Brien